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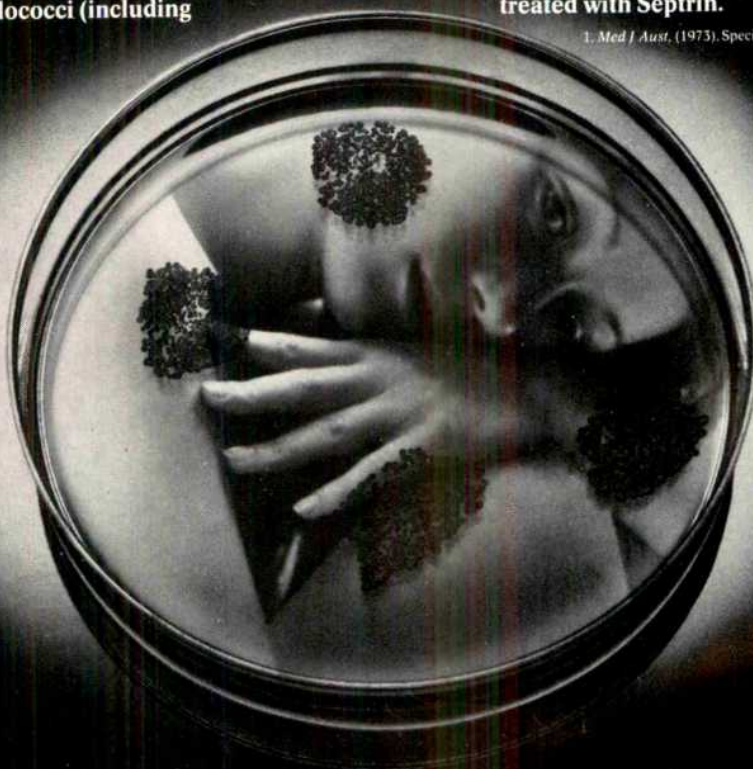
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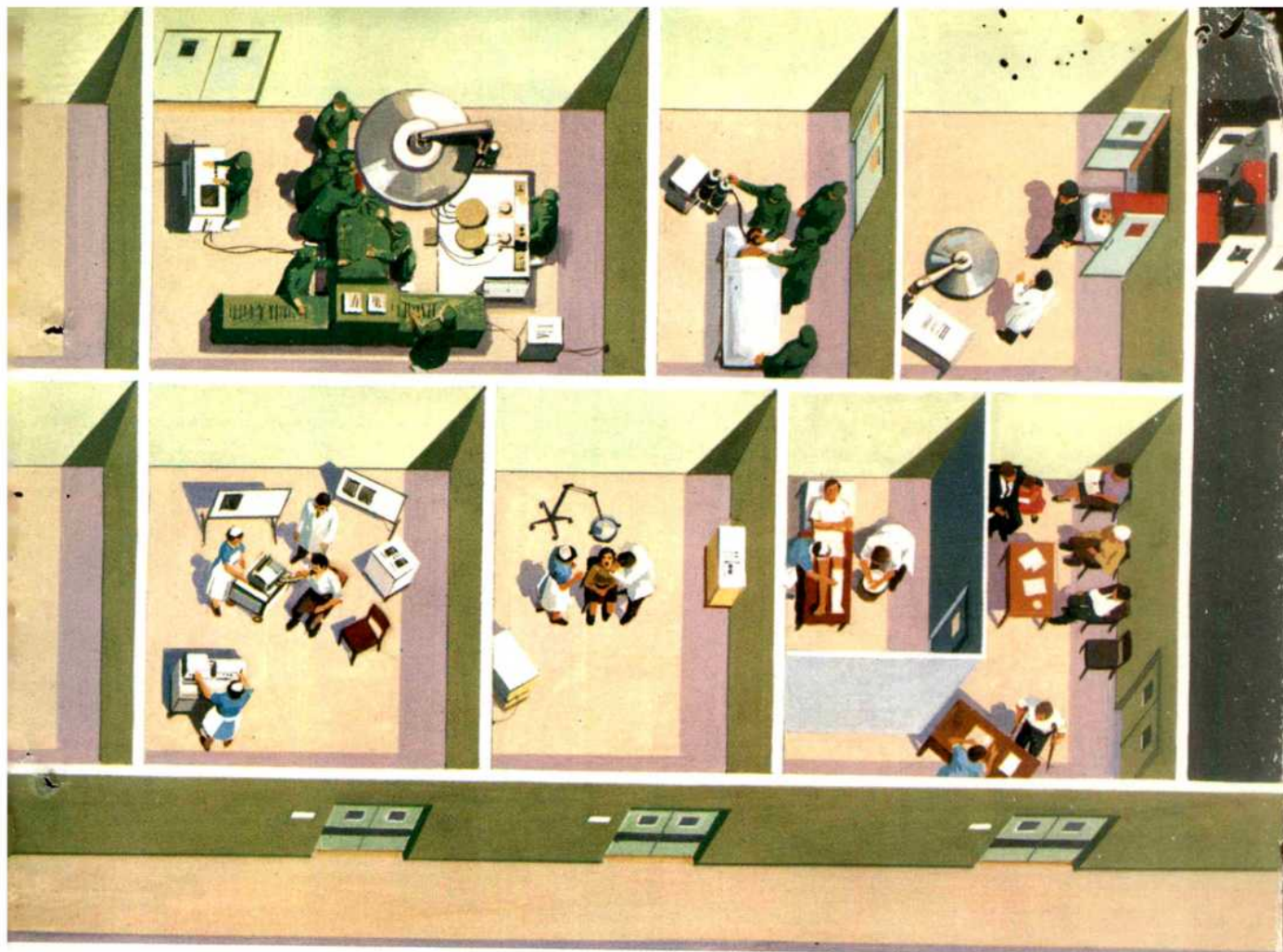
HISTOPATHOLOGY OF THE SKIN

Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosi cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

More than 20 diseases not mentioned in the fourth edition are described in this edition. Some of them are newly recognized entities, such as disseminated superficial actinic porokeratosis, perforating folliculitis, reactive perforating collagenosis, angiolymphoid hyperplasia with eosinophilia, infantile digital fibromatosis, trichilemmoma, and malignant eccrine poroma. Also several previously known diseases because of their increased interest for the dermatologist have been incorporated, among them regional enteritis (Crohn's disease), Tangier disease, lipogranulomatosis (Farber's disease), and generalized eruptive histiocytoma.

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¹ Brit Med J. (1970) 4:455. ² Chemotherapy (1969) 17:1451. ³ Chemotherapy (1969) 17:1496. ⁴ Chemotherapy (1969) 17:1503.
⁵ Clin Trials J. (1970) 7:368. ⁶ Clin Trials J. (1970) 7:375. ⁷ Brit J Clin Pract. (1971) 25:371. ⁸ Chemotherapy (1969) 17:1473.
⁹ Brit J Clin Pract. (1970) 24:41. ¹⁰ Can Med Res Opin. (1975) 3:77. ¹¹ Scand J Infect Dis. (1974) 6:273.
¹² Chemotherapy (1969) 17:1337. ¹³ Chemotherapy (1969) 17:1367. ¹⁴ Chemotherapy (1969) 17:1382.

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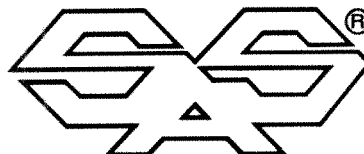
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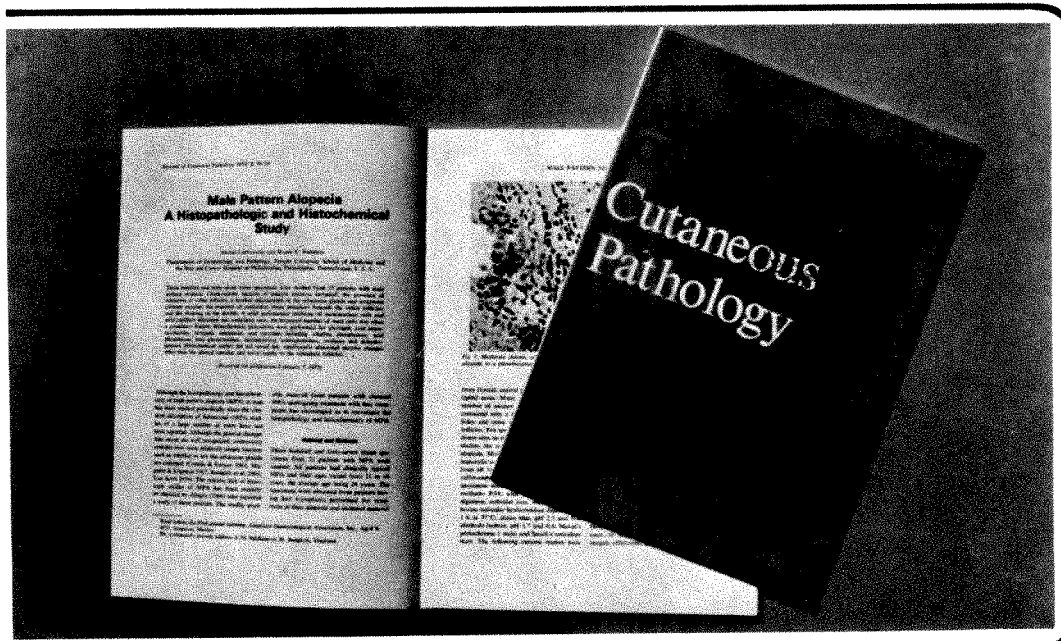


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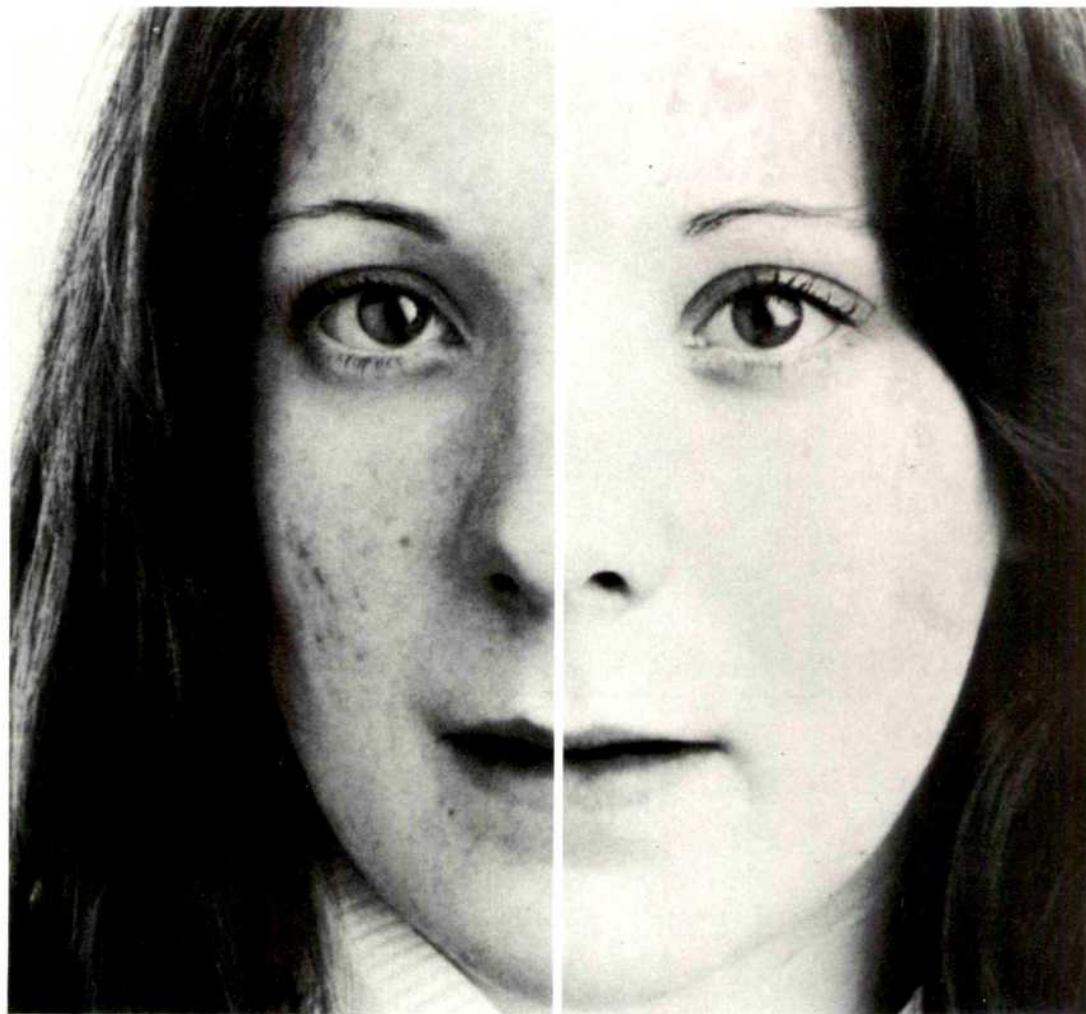
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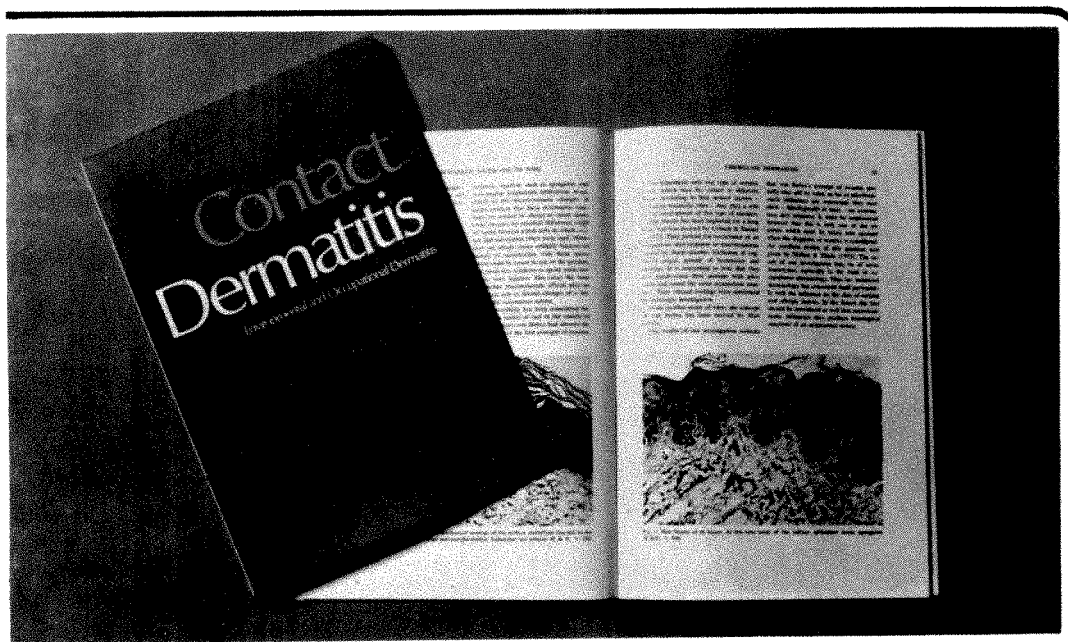
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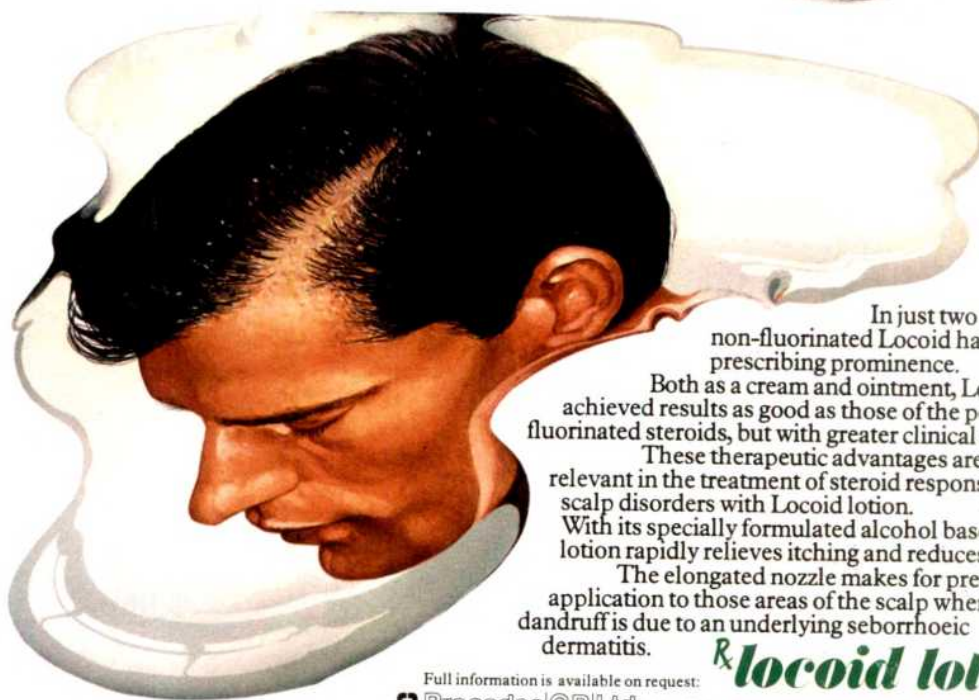
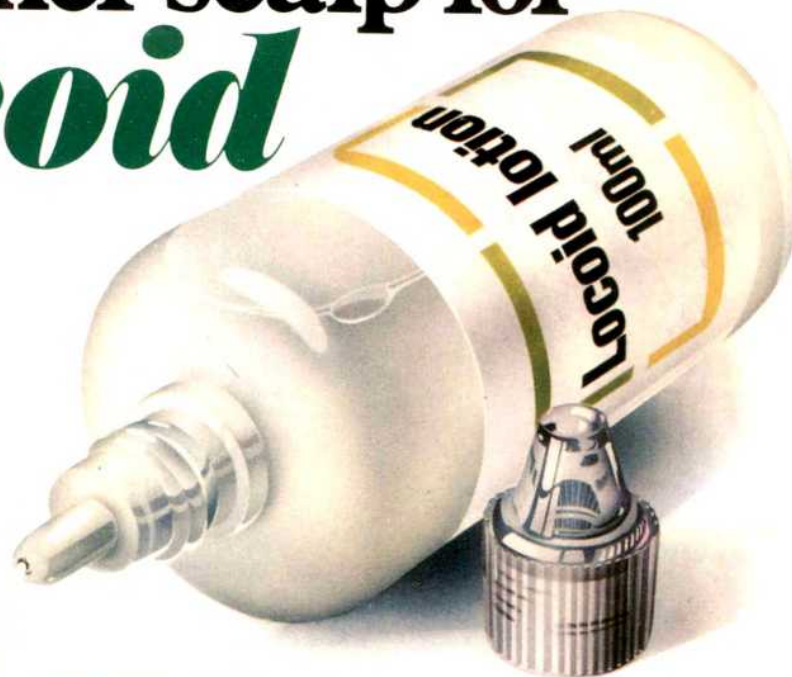
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A concept of diffuse actinic arteritis

THE ROLE OF ACTINIC DAMAGE TO ELASTIN IN 'AGE CHANGE' AND ARTERITIS OF THE TEMPORAL ARTERY AND IN POLYMYALGIA RHEUMATICA

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SUMMARY

Actinic damage (*actinic elastosis*) affecting the internal elastic lamina appears to be the prime cause of 'age change' and arteritis of the temporal artery.

Resorption and removal of altered elastin (elastolysis) is an integral part of the pathology of actinic damage.

Actinic irradiation is probably responsible for the destruction and disappearance of a vast number of arterioles in elastotic skin.

The intimate connection between temporal arteritis and polymyalgia rheumatica prompts the belief that the vascular and other internal malign components of the *temporal arteritis/polymyalgia rheumatica syndrome* might likewise be due, albeit indirectly, to the same actinic cause. Actinic elastotic damage at the body surface could have this effect by provoking a state of systemic elastolysis.

Although ultraviolet (uv) light is often regarded as the sole cause of actinic elastosis, penetrating infrared (heat) irradiation may deserve a large or even a dominant share of the blame.

The obscure origin of temporal artery disease, how and where it begins, has been studied on the basis of two assumptions: (1) That the physical integrity of an artery depends largely on its internal elastic lamina and (2) that the temporal elastic lamina is prone to actinic damage (elastosis), with consequent inflammation and elastolysis (resorption), in much the same manner as pertains to elastic tissue in the dermis.

As regards the first assumption, the indispensability of elastic tissue in vessels is instanced by lathyrism, a nutritional disease in which animals develop aneurysms because the formation of elastic tissue is defective (Barrow, Simpson & Miller, 1974). The stretch resistance of the normal internal elastic lamina has also been confirmed experimentally by Cook, Salmo & Yates (1975). That the lamina plays a dominant role in temporal (giant cell) arteritis was originally stressed by Kimmelstiel, Gilmour &

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Hodges (1952) in their classic paper and has since been reasserted (Heptinstall, Porter & Barkley, 1954; Smith, 1969; Parker *et al.*, 1975).

The second assumption is an extension of the hypothesis of actinic granuloma of the skin, a focal inflammatory disorder that displays persistent nodules and annular lesions (O'Brien, 1975). I have submitted that actinic granuloma is a specific entity in which the degenerate, 'elastotic' fibres of actinically-damaged ('aged') skin (Fig. 1) are resorbed by an *elastolytic* giant cell inflammatory reaction of distinctive pattern (Fig. 2). I have also proposed (O'Brien, 1975, 1976) that actinic granuloma is related to certain disorders affecting African Negroes, namely granuloma multiforme (Allenby & Wilson Jones, 1969) and post-inflammatory elastolysis and cutis laxa (PECL) (Verhagen & Woerdenman, 1975).

In attempting to translate the actinic granuloma hypothesis from skin to vessel it is mandatory first to establish that the temporal artery does in fact suffer actinic damage (actinic elastosis) of essentially the same nature as that found in the skin.

To determine this question, a 'random' series of 100 temporal arteries (right, left or both) was collected from routine autopsies. The subjects were 39 men and 25 women with an age range of 23-91 years.

TECHNIQUE

After marking the deep aspect with Indian ink, the plane of tissue containing the superficial temporal artery in its 'exposed' course across the hairless forehead (Fig. 9) was dissected away from the under surface of the reflected anterior craniotomy flap. Having cross-sectioned the tissue in multiple blocks, the slides were stained by a specially modified haematoxylin-eosin procedure (O'Brien, 1975) to display the characteristically blue 'elastotic' fibres. Special stains, including elastic stains, were also employed.

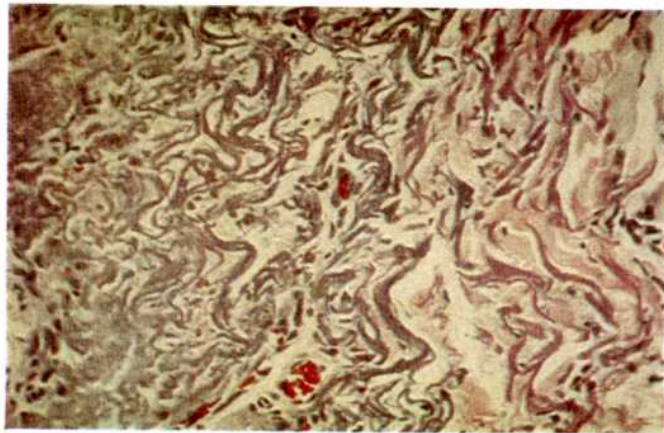
FINDINGS

Only 17 internal elastic laminae in the series appear quite normal throughout their entire circumference in all sections. The remaining 83 show typical actinic elastosis of varying degree, the damaged lamina being distinctly blue and often replicated (Figs 4-7). Even more striking is the selective localisation of the damage within the vessel wall; in 44 arteries the lamina is definitely more elastotic in the 'weather' side of the vessel, that is, in the aspect exposed to the environment, than it is in its deep or cranial side. Often only the 'weather' side is damaged (Fig. 5 and Fig. 9 inset). In no instance does the change appear worse in the deepest lamina. The entire lamina is equally elastotic in 39 and in some of these has been transformed into a jumble of shattered fragments. One vessel displays commencing inflammatory infiltration and is thrombosed (Fig. 7). In many, the lamina has undergone elastolysis in whole or in part, again with a bias for the 'exposed' aspect (Figs 6 and 7).

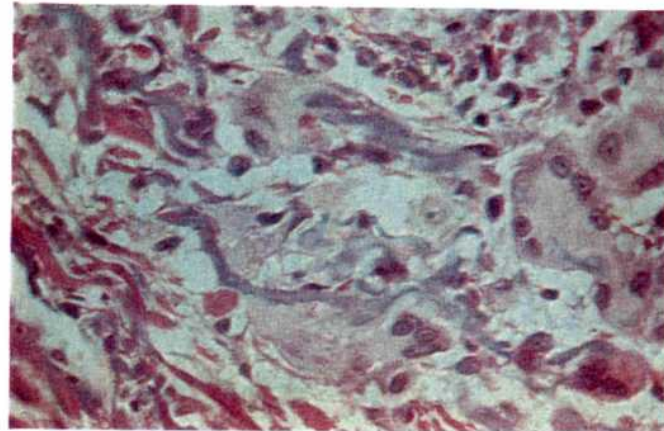
The disorder of the lamina reveals a simple time gradient, the oldest subjects having the most damage. A definite age of onset cannot be established from this study but, of the 8 subjects less than 50 years old, 4 show frank elastosis. The earliest detectable change is in a 31-year-old male.

According to my findings, actinic elastosis of the lamina precedes and merges into 'age change' of the temporal artery as described by Ainsworth, Gresham & Balmforth (1961) and by Lie, Brown & Carter (1970). 'Age change' is the broad term commonly used to denote all the degenerative and proliferative reactions that may come to impair both the muscle and the elastic tissue of the artery. In the end, fibrosis tends to dominate.*

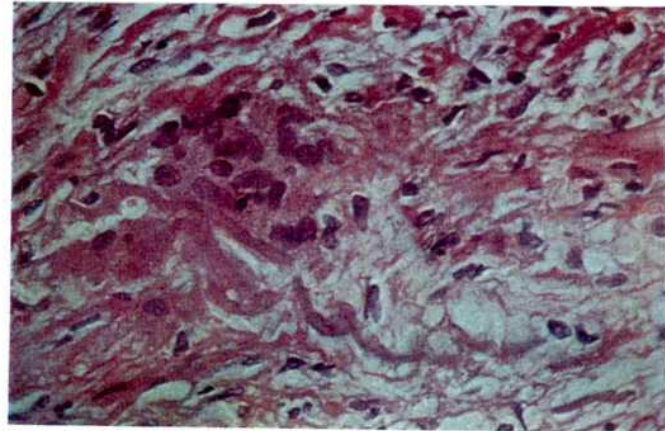
* Ainsworth *et al.* (1961) and Lie *et al.* (1970) did not describe actinic elastosis of the lamina. This disparity could be due to different staining techniques. Also, as stated at the outset, my investigation was undertaken specifically to assess the presence and role of actinic elastosis in the artery.



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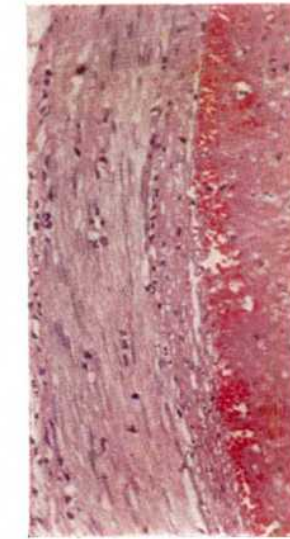
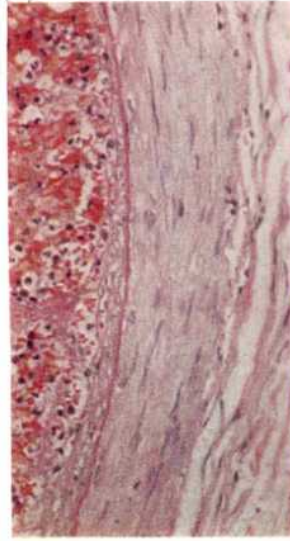


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FIGURE 1. Actinic elastosis of skin. A picture of the entire dermis showing the classic appearance of dense masses of blue-staining, degenerate (elastotic) fibres. The blue masses extend deeply; the deepest fibres are stained red and are probably in a hyperplastic phase. Note the paucity of arterioles. (Dermis from sun-protected areas normally stains a uniform pink and its elastic fibres do not stain selectively) (H & E, $\times 300$).

FIGURE 2. Actinic granuloma of skin. Fragments of thick, blue, actinically-damaged (elastotic) fibre inside giant cells. Digestion (elastolysis) is probably occurring (H & E, $\times 500$).

FIGURE 3. Temporal (giant cell) arteritis. Thick, broken bluish (elastotic) internal elastic lamina with adjacent giant cell. (Normally the lamina stains a bright red with my technique) (H & E, $\times 500$).



8

7b

FIGURE 4. Actinic elastosis in the temporal artery. Random autopsy, female aged 52. Early blue change is present in the outermost, 'exposed' or 'weather' aspect of the lamina (above). Normal, red, deep elastic lamina (below). The wavy appearance of the lamina is normal in sections. (Compare Fig. 9 Inset) (H & E, $\times 300$).

FIGURE 5. Actinic elastosis in the temporal artery. Another random autopsy, female aged 53. The lamina of the 'exposed' or 'weather' wall (above) is blue, thick, replicated, ? broken. Note thickened intima. Unaffected red lamina (below) (H & E, $\times 300$).

FIGURE 6. Another section of the same temporal artery as Fig. 5. Marked changes are seen in the outer 'weather' wall: irregular blue lamina with evidence of resorption, replication and fibrosis (confirmed by special stains). Normal red lamina below (H & E, $\times 150$).

FIGURE 7. Temporal artery. Random autopsy, male aged 47. Two high-power views showing the contrast between the damaged, outer lamina and the normal, deep lamina. (a) The damaged elastotic lamina crosses under the muscle just below mid-picture and consists of thick, blue, separated fragments. Some neutrophils are seen near the fragments and also further out, in the muscle. (b) The normal lamina is stretched but quite intact and stains a normal red. No infiltration is seen near the lamina or elsewhere. Note thrombus in lumen in both pictures (H & E, $\times 300$).

FIGURE 8. Temporal artery. Random autopsy. This picture shows calcification and illustrates how it differs from actinic elastotic damage of the lamina. The blue calcific deposit surrounds the lamina whilst the lamina itself stains red and appears quite normal (H & E, $\times 300$).

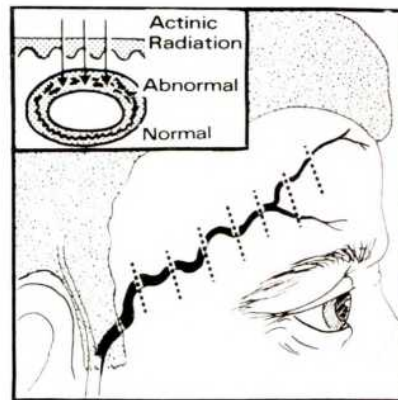


FIGURE 9. The anterior branch of the superficial temporal artery in its unprotected course across forehead. Section sites marked. INSET. Section illustrating greater irradiation and more damage to the elastic lamina of outer 'exposed' or 'weather' wall.

Calcification is quite rare in the series and affects only 6 arteries as blue-staining deposits on and near, but not in, the lamina (Fig. 8). Calcification may occur independently of elastosis and, whilst more study is required, I regard it as incidental and of little consequence for the artery. In this respect, calcification seems akin to that of Mönckeberg's medial calcific sclerosis (Silbert, Lippmann & Gordon, 1953).

In a supplementary series, 70 arteries from the hair-covered, partly sun-shaded scalp have been found to display conspicuously less actinic damage than the 'exposed' temporals from the same subjects. Again the damage is more intense in the superficial elastica.

Special stains

The actinic causation of blue elastosis has been acknowledged by Rook & Wilkinson (1972), and is well expressed by Pathak & Epstein (1971) who state: 'The most conspicuous and characteristic structural change in the skin involving solar degeneration is an alteration of connective tissue so that it appears strikingly basophilic in sections stained with hematoxylin and eosin.' I concur with this statement and believe that, if fresh Harris haematoxylin and buffered watery eosin Y are used (O'Brien, 1975), blue staining of elastin is virtually specific for actinic damage, only calcification needing to be excluded. The blue staining property of laminal elastosis is not abolished by pretreatment with acid, a finding that supports its distinction from calcification. Elastic stains have not contributed additional information of aetiological value in this study but have simply confirmed elastotic replication or elastolysis when these have already been suspected from the haematoxylin.

Elastotic actinic damage and elastolysis of cutaneous vessels

The remarkable paucity of arterioles within severely sun-damaged ('aged') skin is currently being investigated with the benefit of knowledge provided from the study of the temporal artery, this being the 'type' model. A brief summary is included here.

Whereas the temporal artery is a substantial vessel and large enough to survive actinic damage, if only to become a blocked and useless cord, the same is not true of dermal vessels, many of which disappear entirely (Fig. 1) and are replaced by the familiar, scanty telangiectases. The process of erasure of vessels probably takes place gradually throughout the life of the individual and is often difficult to appreciate histologically. Nevertheless, I propose that there are two main modes of erasure. On the

one hand, vessels may 'fade out' in a slow manner that seems equivalent to 'ageing' in the temporal artery. Prior to final exit, such 'ex-vessels' may persist for long periods in the superficial dermis as hyaline, elastotic strands. Alternatively, destruction may cause so much infiltrate, so much tissue commotion, that the disrupting vessel cannot be recognized at all. This form of exit may be equivalent to active temporal arteritis.

Although a difficult matter to prove, it seems likely that the actinic destruction of vessels sometimes induces the necrobiosis found in a variety of conditions such as 'atypical' necrobiosis lipoidica and actinic granuloma.

DISCUSSION

Kinmont & McCallum (1964a, b) were the first observers to implicate actinic radiation in the aetiology of temporal arteritis/polymyalgia rheumatica (Fig. 10). Having quoted a striking series of clinical histories, including that of an invalid ex-farmer who developed temporal arteritis soon after a month of exposure to the 'brilliant' English sunshine of May 1964, Kinmont & McCallum (1964b) went on to conclude: 'In only four of the eighteen cases that we were able to examine was there no history of undue exposure to light or light sensitivity'. Later in the same paper they proposed 'some degeneration factor produced in the skin by ultra-violet light' (my italics). Here they seem to have been almost on the verge of specifying actinic elastosis/elastolysis. Elsewhere, they spoke of 'collagen degeneration', a

POLYMYALGIA RHEUMATICA: Clinical Aspects.

A CRIPPLING DISORDER AFFECTING PERSONS AGED 50 AND OVER.
 POORLY-RECOGNIZED YET DEFINITE ENTITY. NOT RARE
 MAY BE SECONDARY TO OTHER DISORDERS e.g. COLLAGEN DISEASE
 MALIGNANCY
 INFECTIONS

PAIN



HERE

THERE

EVERYWHERE

SYMPTOMS:

- RHEUMATIC PAINS AND STIFFNESS (INTENSE, DIFFUSE, MAINLY LIMB GIRDLES)
- GENERAL MALAISE

SIGNS:

- SCANTY AND VAGUE
- MILD ANAEMIA

DIAGNOSIS:

- VERY HIGH E.S.R. (OFTEN 100 mm OR MORE, WESTERNGREN)
- RAISED α_2 GLOBULIN IN SERUM
- TEMPORAL ARTERY BIOPSY (? ARTERITIS)

COMPLICATIONS:

- ANEURYSM AND/OR OCCLUSION OF VESSELS e.g. AORTA, OPHTHALMIC ARTERY (BLINDNESS)

THERAPY:

- DRAMATIC RELIEF WITH CORTICOSTEROIDS

FIGURE 10. Polymyalgia rheumatica: A summary of the chief features of the disease.

term then often used for elastosis, and elsewhere again, they stated that all 18 cases had senile purpura, a kindred phenomenon.

My own study of actinic elastosis and elastolysis in the temporal artery has led me, independently and by a different route, to essentially the same conclusion as reached by Kinmont & McCallum, namely, that actinic damage is the basis of temporal arteritis/polymyalgia. Moreover, elastotic actinic damage, and particularly its involvement of vascular laminae, seems to supply the *modus operandi* for fulfilling their clinical prediction. It also meets the requirement of the aetiological summary by Robbins (1974) for 'a *peculiar* degenerative change in the elastica' (my italics).

The typical blue elastosis in the temporal lamina and its distinctly biased incidence in the exposed or 'weather' aspect of the artery support the belief that the lamina, like elastic tissue in the skin, is extremely susceptible to damage by penetrating actinic (radiant) energy. The resulting blue elastosis in the vessel has exactly the same microscopic appearance as that seen in the dermis. At the same time, blue elastosis as it affects the vessel appears unique amongst vascular lesions and, in my experience, differs sharply from currently accepted vascular entities, whether occurring in comparable sun-protected sites or deep within internal tissues. Any supposition that the blue change could be primarily mechanical rather than radiant therefore does not appear valid. For these reasons, and because of the weight of collateral evidence annotated elsewhere in this paper, I submit that blue laminal degeneration should be regarded as *actinic* until proved otherwise.

A further proposal I wish to make is that actinic laminal elastosis provokes the auto-aggressive inflammation of temporal arteritis just as the same type of damage in the dermis seems to provoke actinic granuloma in the skin (O'Brien, 1975). This proposal is a specification of the broader statement of Mowat & Hazleman (1974) 'that the arteritis is an *acute allergic reaction* to the internal elastic lamina which has been damaged *with advancing age*.' (my italics). An additional disruptive factor acting upon an actinically-weakened artery is the unremitting pulse and pressure stress and this seems likely to exacerbate both the damage and the inflammation.

According to my interpretation, 'age change' and arteritis of the temporal artery are closely related and either condition may arise during the individual's lifespan according to the balance struck between continuing actinic damage, granulomatous inflammation, thrombosis and repair. In support of this interpretation, my staining procedure has demonstrated segments of blue-staining lamina in 'aged' (abnormal but not inflamed) arteries (Figs 4-7), and blue fragments have constantly been found inside and outside some of the giant cells in active temporal arteritis (Fig. 3). An instance of commencing inflammatory infiltration in relation to elastotic fragments was encountered in the 'random' series (Fig. 7).

Systemic vascular disease and elastolysis in polymyalgia rheumatica

In their review, Hamilton *et al.* (1971) concluded that temporal arteritis and polymyalgia are manifestations of one and the same systemic disorder and, like Kinmont & McCallum (1964a, b) used the term giant cell arteritis to include both conditions. Therefore it seems legitimate to propose a common pathogenesis related to actinic elastosis/elastolysis, as follows:

Hypothesis. The phenomenon of elastolysis that occurs in actinic granuloma and temporal arteritis gives a suggestive lead as to the pathogenesis of polymyalgia. Instead of a slow, localized phagocytic type of reaction as seen in granuloma and arteritis, I propose that the elastolysis of polymyalgia is acute, diffuse and humoral. Moreover, whilst the 'antigen' is likely to be the same, namely the large accumulations of actinically damaged fibres in the skin and superficial vessels, systemic overflow of anti-elastic activity may take place in polymyalgia with the result that *normal* elastin in internal tissues is also consumed. Because of their dependence on elastin, vessels everywhere, and particularly the great vessels, suffer the most damage (Fig. 11).

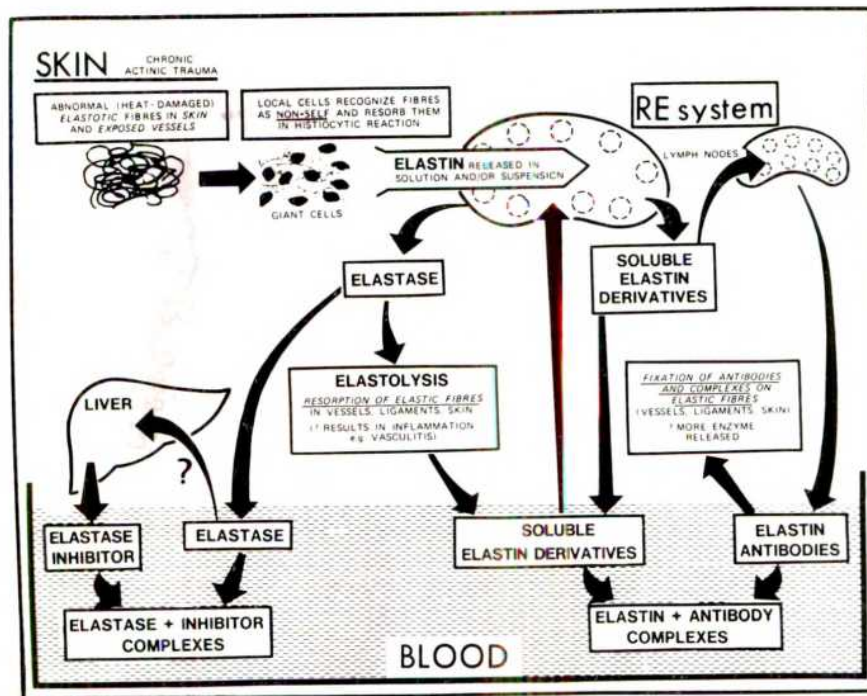


FIGURE 11. An actinic elastosis/elastolysis hypothesis of polymyalgia rheumatica: The top of the schema indicates the conjectured, long preparatory stage of polymyalgia during which the body builds up auto-immunity against actinically-damaged elastin. The rest of the schema portrays the cycle of events suspected to occur in the acute crisis of polymyalgia, with increasing amounts of released elastin derivatives stimulating more and more elastolytic enzymes and anti-elastin antibodies.

The identity of the elastase(s) is unknown but a connective tissue rather than a pancreatic origin seems likely. A moderating influence by elastase inhibitor (α_1 antitrypsin) is shown. Secondary auto-immune reactions involving collagen and smooth muscle could arise but are not depicted.

This auto-immune pathogenesis is compatible with the views of Mowat & Hazleman (1974) and with the actinic hypothesis of Kinmont & McCallum (1964a, b). It is also consistent with some specific observations that puzzled Kinmont & McCallum, namely the 'sarcoidosis' (1964a) and the 'mysterious allergic eruption of the face, neck and hands during the spring and summer' (1964b). I believe that these lesions and others like them may have been instances of actinic granuloma or its sarcoid variant. In a series of 25 cases of polymyalgia I have confirmed the frequent presence of clinically severe actinic elastosis/elastolysis, often in freckled, 'Celtic' skin. Whilst I have found no frank clinical granulomas, microscopic elastolytic granulomas have been detected in two of four skin biopsies.

Further support for the hypothesis comes from other sources, one of the most interesting and significant being in reports of certain African children who suffer from dermal granulomas, acquired cutis laxa (premature ageing) and internal vascular and pulmonary lesions (Marshall, Heyl & Weber, 1966; Heyl, Simson & Cronje, 1971; Verhagen & Woerdeman, 1975). Although other explanations have been advocated (Verhagen & Woerdeman, 1975), I believe (O'Brien, 1976) that these African reports are best understood if we suppose that the children were suffering from a genetic defect in their elastic fibres which made them acutely sensitive to actinic elastotic damage and to its granulomatous and

systemic elastolytic consequences. Indeed, such children seem to duplicate, in an exaggerated and bizarre form, the 'senile' (lax) skin and internal elastolytic lesions of adult polymyalgia.

Immunological evidence that temporal arteritis/polymyalgia is elastin-related comes from the work of Liang, Simkin & Mannik (1974) who demonstrated apparent anti-elastin antibody deposited upon the elastic lamina in temporal arteritis. Using the lymphocyte transformation test, Hazleman, MacLennan & Esiri (1975) have demonstrated that lymphocytes from polymyalgia patients react to an elastin-containing arterial antigen.

Direct evidence of the occurrence of systemic elastolysis in polymyalgia is currently being sought in my own laboratory by a different approach. Because elastic/elastotic fibres and their derivatives are fluorescent (Quinn & Blout, 1970; Partridge, 1970; Jarrett, 1974a; La Bella, 1974) this study is based on the assumption that widespread resorption of fibres might induce fluorescence of the serum and reticulo-endothelial cells in polymyalgia. Some confirmation of this expectation has been found; the α_2 band in electrophoresis strips prepared from polymyalgia sera frequently develops an apparently selective fluorescence if the strips are stored in the dark for some weeks. Strips prepared from normal and various control sera do not display α_2 fluorescence to the same degree. This study which is based on 100 sera will be reported separately.

Elastolysis wherever it occurs is probably a function of proteolytic enzymes and tissue elastases. Elastases are well recognized in blood leukocytes (Janoff, 1972; Ohlsson & Olsson, 1974) and in platelets (Legrand *et al.*, 1973). Cathepsins are another group of enzymes capable of digesting elastin (Greenbaum, 1971; Dingle, Barrett & Weston, 1971).

Elastases are believed to be involved in some varieties of emphysema wherein the elastic framework of the lung is attacked and resorbed because of a genetic deficiency of anti-elastase or α_1 antitrypsin (Laurell, 1971; Blackwood *et al.*, 1974; Lieberman, 1976). A notion of delicate balance between elastase and anti-elastase activity in emphysema has been described by Laurell (1971) and seems to be also applicable, in a broader sense, to polymyalgia. Another interesting possibility is that the poorly defined pulmonary signs of polymyalgia (Kinmont & McCallum, 1964b; Hamilton *et al.*, 1971) could themselves be elastolytic in origin. Here we should recall that a link is known to exist between 'senile' skin and emphysema in 50–70-year-old males (Smith, Williamson & McConkey, 1967).

It is apparent that the immunological and enzymatic aspects of polymyalgia are challenging and that research into them must proceed despite the obstacles presented by the insolubility of elastin (Partridge & Keeley, 1974). Even more provocative is the emerging realization that the actinic anti-elastin hypothesis of polymyalgia takes us remarkably close to the auto-immune theories recently proposed for atherosclerosis (Robert & Robert, 1973; Mathews, Whittingham & Mackay, 1974).

The nature of actinic elastosis

Actinic elastosis, often referred to as 'solar', 'senile' or 'basophilic' degeneration, is generally accepted as responsible for the retrogressive 'age changes' that occur in the dermis of exposed skin (Jarrett, 1974b; Ledoux-Corbusier & Achten, 1974). Possessing a significance that passes beyond the mere cosmetic, the subject requires consideration here.

Some of the problems and uncertainties concerning elastosis have been compounded by the use of inadequately-controlled staining methods. It should also be recognized that optimum conditions for the study of actinic damage and its sequelae are found in hot, sunny climates.

With a suitable technique, actinically-damaged dermis typically displays a gross excess of elastic fibres that differ from normal in as much as they attract the blue haematoxylin stain—thus the attributes 'basophilic' and 'elastotic' (Fig. 1). Two phases of actinic elastosis probably exist. The first, seen at the bottom of Fig. 1, is a simple hyperplasia of normal or near-normal fibres; this presumably arises from the radiant stimulation of fibroblasts. The second and more familiar phase of blue degeneration

seems to develop outside the cells after the fibres have been formed. Whereas the 2 phases commonly co-exist, at least in the dermis, study of the internal elastic lamina indicates that degeneration may occur without prior hyperplasia. It is possible that the two phases of elastosis are caused by different components of the solar spectrum.

The reason why elastic tissue responds to irradiation in such a peculiar fashion is unknown. So too is the chemical basis of the blue change. The *in situ* degeneration of the lamina confirms that the blue change is intrinsic within elastic tissue and that collagen does not contribute directly, as some have believed.

The total accumulation of elastotic fibres at the body surface may become immense. This is because the skin is the body's most massive single organ and consists almost entirely of dermis that is subject to diffuse elastosis.

From a functional point of view it seems that elastotic fibres are not only fragile but are particularly liable to partial or complete resorption, a process that might be called *actinic elastolysis*. Although often subtle, actinic elastolysis appears to be a pathological process of fundamental significance and, despite views to the contrary (La Bella, 1974), is by no means rare. One of the few pertinent experimental facts known about it is that actinic (uv) exposure increases the susceptibility of elastin to elastase digestion (La Bella, 1974). The size of elastotic deposits probably influences both the excitation of elastolysis as well as its effects.

The aetiology of elastotic/elastolytic 'ageing' is a vexed question, the only consensus being that it results from exposure to radiant energy. Because of a known epithelial impact, short-wave uv light has often been indicted as responsible. In 1964 it was natural for Kinmont & McCallum (1964a, b) to specify 'ultra-violet light' as the damaging actinic component in polymyalgia and to use the term 'light sensitivity' throughout their papers. This emphasis on light may have contributed to the unfortunate, almost complete neglect and discard of their actinic hypothesis, as many students of temporal arteritis have hesitated to accept that light could penetrate so deeply and inflict such destruction. These reservations about light have since been justified, Kligman (1974) stating that it has repeatedly been confirmed that very little short-wave (erythemic) light penetrates beyond the surface epithelium of (white) skin. Long-wave uv penetrates deeper (Kligman, 1974) and, whilst it can produce photo-sensitivity reactions (Ryan, 1972) and augment the erythemic effects of short-wave uv (Willis, Kligman & Epstein, 1973), there is no satisfying direct evidence that it can elicit elastosis of any kind in man, let alone elastosis at the degenerative (blue) phase.*

Perhaps the solution to the enigma of actinic damage in polymyalgia resides in the pathogenicity of infrared (heat) irradiation, for this wave-band is known to cause elastosis in *erythema ab igne* (Finlayson, Sams & Smith, 1966). Strangely, the significance of this generally accepted finding has not been fully appreciated or its implications translated to other parts of the body that are exposed to intense infrared from the sun and other sources. I therefore propose that infrared irradiation should be recognized as a potent if not the dominant agent of elastosis/elastolysis in all areas of the integument and add the following data in support:

- (1) Infrared irradiation penetrates readily and deeply and can doubtless reach the temporal artery (Forsythe & Adams, 1944).
- (2) That it penetrates into the hair-covered scalp is a common personal experience. Such penetration could explain how scalp vessels suffer actinic damage, although to a lesser degree than the 'naked' temporal artery.
- (3) The penetrating, damaging properties of infrared could help us understand why the black skin

* In experimental work on elastosis, heat generated by light sources needs to be taken into account. The suitability of animals for studies of this kind has not been established.

of the Negro is not immune to actinic elastosis (Kligman, 1974) or to the elastolytic sequelae that I have suggested (1975, 1976) may flow from it, namely granuloma multiforme and post-inflammatory cutis laxa.

(4) The preferential incidence of temporal arteritis/polymyalgia detected amongst females in some studies (Hamilton *et al.*, 1971) may be related to particular sources of infrared generation such as domestic stoves and radiant heat hair-dryers.

(5) Instances of probable thermal aggravation of elastosis/elastolysis by occupation have been reported in the literature. As long ago as 1884, Rossbach (quoted by Goltz *et al.*, 1965) proposed that the heat from an oven aggravated the cutis laxa of a baker. McCarthy, Warin & Read (1965) described the case of a 'fireman' with cutis laxa whose condition was apparently worse on the exposed areas of the body and was accompanied at those sites by giant cell granulomas. I suspect from the description that at least some of the granulomas were elastolytic, like actinic granuloma. Reed, Horowitz & Beighton (1971) reported an instance of acquired cutis laxa in an 'engineer', another often hot occupation.

(6) One of the most suggestive pieces of indirect evidence comes from the work of Mowat & Hazleman (1974). Apparently unaware of Kinmont and McCallum's contribution, these observers confirmed the summer peak of polymyalgia and, in addition, detected an even higher peak in winter. This fresh emphasis on heat in summer prompts the question as to how an actinic factor might operate so strongly in winter. In the English climate, light could hardly be responsible; could it not be heat from domestic radiant sources, a kind of *systemic erythema ab igne*?

In summary, it may be concluded that the identity of the wave-band or bands responsible for connective tissue damage is by no means certain and that infrared irradiation should be given due recognition amongst the possible causes.

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REFERENCES

- AINSWORTH, R.W., GRESHAM, G.A. & BALMFORTH, G.V. (1961) Pathological changes in temporal arteries removed from unselected cadavers. *Journal of Clinical Pathology*, **14**, 115.
- ALLENBY, C.F. & WILSON JONES, E. (1969) Granuloma multiforme. *Transactions of the St John's Hospital Dermatological Society*, **55**, 88.
- BARROW, M.V., SIMPSON, C.F. & MILLER E.J. (1974) Lathyrism: a review. *Quarterly Review of Biology*, **49**, 101.
- BLACKWOOD, C.E., MORET, J.E., KELLER, S., FIERER, J.A. & MANDL, I. (1974) Alpha₁-antitrypsin concentration in serum of laboratory animals. Changes following proteolytic enzyme injection. *Journal of Laboratory and Clinical Medicine*, **84**, 813.
- COOK, T.A., SALMO, N.A.M. & YATES, P.O. (1975) The elasticity of the internal lamina. *Journal of Pathology*, **117**, 253.
- DINGLE, J.T., BARRETT, A.J. & WESTON, P.D. (1971) Cathepsin D. Characteristics of immunoinhibition and the confirmation of a role in cartilage breakdown. *Biochemical Journal*, **123**, 1.
- FINLAYSON, G.R., SAMS, W.M. & SMITH, J.G. (1966) Erythema ab igne: A histopathological study. *Journal of Investigative Dermatology*, **46**, 104.
- FORSYTHE, W.E. & ADAMS, E.Q. (1944) Radiation: Sources of ultraviolet and infra-red. In: *Medical Physics* (Ed. by O.Glasser), Vol. 1, p.1157. The Year Book Publishers, Chicago.
- GOLTZ, R.W., HULT, A.-M., GOLDFARB, M. & GORLIN, R.J. (1965) Cutis laxa. A manifestation of generalized elastolysis. *Archives of Dermatology*, **92**, 373.
- GREENBAUM, L.M. (1971) Cathepsins and kinin-forming and -destroying enzymes. In: *The Enzymes* (Ed. by P.D. Boyer), 3rd edn, Vol. III, Chapter 12, p.475. Academic Press, New York and London.

- HAMILTON, C.R., SHELLEY, W.M. & TUMULTY, P.A. (1971) Giant cell arteritis: including temporal arteritis and polymyalgia rheumatica. *Medicine (Baltimore)*, **50**, 1.
- HAZLEMAN, B.L., MACLENNAN, I.C.M. & ESIRI, M.M. (1975) Lymphocyte proliferation to artery antigen as a positive diagnostic test in polymyalgia rheumatica. *Annals of Rheumatic Diseases*, **34**, 122.
- HEPTINSTALL, R.H., PORTER, K.A. & BARKLEY, H. (1954) Giant cell (temporal) arteritis. *Journal of Pathology and Bacteriology*, **67**, 507.
- HEYL, T., SIMSON, I.W. & CRONJE, R.E. (1971) Post-inflammatory cutis laxa and aortitis (Acquired systemic elastolysis). *British Journal of Dermatology*, **85**, Suppl. 7, 37.
- JANOFF, A. (1972) Human granulocyte elastase. *American Journal of Pathology*, **68**, 579.
- JARRETT, A. (1974a) The elastic tissue of the dermis. In: *The Physiology and Pathophysiology of the Skin* (Ed. by A.Jarrett), Vol. 3, Chapter 23, p.847. Academic Press, London.
- JARRETT, A. (1974b) Cutaneous elastoses. In: *The Physiology and Pathophysiology of the Skin* (Ed. by A.Jarrett) Vol. 3, Chapter 28, p.973. Academic Press, London.
- KIMMELSTIEL, P., GILMOUR, M.T. & HODGES, H.H. (1952) Degeneration of elastic fibers in granulomatous giant cell arteritis (temporal arteritis). *Archives of Pathology*, **54**, 157.
- KINMONT, P.D.C. & MCCALLUM, D.I. (1964a) Skin manifestations of giant-cell arteritis. *British Journal of Dermatology*, **76**, 299.
- KINMONT, P.D.C. & MCCALLUM, D.I. (1964b) The aetiology, pathology and course of giant-cell arteritis. The possible role of light sensitivity. *British Journal of Dermatology*, **77**, 193.
- KLIGMAN, A.M. (1974) Solar elastosis in relation to pigmentation. In: *Sunlight and Man. Normal and Abnormal Photobiologic Responses* (Ed. by T.B.Fitzpatrick, M.A.Pathak, L.C.Harber, M.Seiji and A.Kukita), p.157. University of Tokyo Press, Japan.
- LABELLA, F.S. (1974) Enzymic vs non-enzymic factors in the deterioration of connective tissue. *Advances in Experimental Medicine and Biology*, **43**, 377.
- LAURELL, C.B. (1971) Is emphysema in alpha₁-antitrypsin deficiency a result of autodigestion? *Scandinavian Journal of Clinical and Laboratory Investigation*, **28**, 1.
- LEDoux-CORBUSIER, M. & ACHTEN, G. (1974) Elastosis in chronic radiodermatitis. An ultrastructural study. *British Journal of Dermatology*, **91**, 287.
- LEGRAND, Y., CAEN, J., BOOYSE, F.M., RAFELSON, M.E., ROBERT, B. & ROBERT, L. (1973) Studies on a human blood platelet protease with elastolytic activity. *Biochimica et Biophysica Acta*, **309**, 406.
- LIANG, G.C., SIMKIN, P.A. & MANNIK, M. (1974) Immunoglobulins in temporal arteries. An immunofluorescent study. *Annals of Internal Medicine*, **81**, 19.
- LIE, J.T., BROWN, A.L. & CARTER, E.T. (1970) Spectrum of aging changes in temporal arteries. Its significance, in interpretation of biopsy of temporal artery. *Archives of Pathology*, **90**, 278.
- LIEBERMAN, J. (1976) Elastase, collagenase, emphysema and alpha₁-antitrypsin deficiency. *Chest*, **70**, 62.
- MARSHALL, J., HEYL, T. & WEBER, H.W. (1966) Postinflammatory elastolysis and cutis laxa. *South African Medical Journal*, **40**, 1016.
- MATHEWS, J.D., WHITTINGHAM, S. & MACKAY, I.R. (1974) Autoimmune mechanisms in human vascular disease. *Lancet*, **ii**, 1423.
- MCCARTHY, C.F., WARIN, R.P. & READ, A.E.A. (1965) Loose skin (cutis laxa) associated with systemic abnormalities. *Archives of Internal Medicine*, **115**, 62.
- MOWAT, A.G. & HAZLEMAN, B.L. (1974) Polymyalgia rheumatica—a clinical study with particular reference to arterial disease. *Journal of Rheumatology*, **1**, 190.
- O'BRIEN, J.P. (1975) Actinic granuloma. *Archives of Dermatology*, **111**, 460.
- O'BRIEN, J.P. (1976) Is actinic (solar) damage the provoking cause of 'post-inflammatory elastolysis and cutis laxa (PECL)'? *British Journal of Dermatology*, **95**, 105.
- OHLSSON, K. & OLSSON, I. (1974) The neutral proteases of human granulocytes. Isolation and partial characterization of granulocyte elastases. *European Journal of Biochemistry*, **42**, 519.
- PARKER, F., HEALEY, L.A., WILSKE, K.R. & ODLAND, G.F. (1975) Light and electron microscopic studies on human temporal arteries with special reference to alterations related to senescence, atherosclerosis and giant cell arteritis. *American Journal of Pathology*, **79**, 57.
- PARTRIDGE, S.M. (1970) Biological role of cutaneous elastin. In: *Advances in Biology of Skin. The Dermis* (Ed. by W.Montagna, J.P.Bentley and R.L.Dobson), Vol. X, Chapter V, p.69. Appleton-Century Crofts, New York.
- PARTRIDGE, S.M. & KEELEY, F.W. (1974) Age related and atherosclerotic changes in aortic elastin. *Advances in Experimental Medicine and Biology*, **43**, 173.

- PATHAK, M.A. & EPSTEIN, J.H. (1971) Normal and abnormal reactions of man to light. In: *Dermatology in General Medicine* (Ed. by T.B.Fitzpatrick, K.A.Arndt, W.H.Clark, A.Z.Eisen, E.J.Van Scott and J.H.Vaughan), p.977. McGraw-Hill, New York.
- QUINN, R.S. & BLOUT, E.R. (1970) Spectrofluorometric assay for elastolytic enzymes. *Biochemical and Biophysical Research Communications*, **40**, 328.
- REED, W.B., HOROWITZ, R.E. & BEIGHTON, P. (1971) Acquired cutis laxa. Primary generalized elastolysis. *Archives of Dermatology*, **103**, 661.
- ROBBINS, S.L. (1974) Giant cell arteritis (temporal arteritis, cranial arteritis). In: *Pathologic Basis of Disease* (Ed. by S.L.Robbins), p.605. W.B.Saunders, Philadelphia.
- ROBERT, L. & ROBERT, B. (1973) Immunology and aging. *Gerontologia*, **19**, 330.
- ROOK, A. & WILKINSON, D.S. (1972) The principles of diagnosis. In: *Textbook of Dermatology* (Ed. by A.Rook, D.S.Wilkinson and F.J.G.Ebling), 2nd edn, p.37. Blackwell Scientific Publications, Oxford.
- RYAN, T.J. (1972) Cutaneous reactions to actinic and ionizing radiations. In: *Textbook of Dermatology* (Ed. by A.Rook, D.S.Wilkinson and F.J.G.Ebling), 2nd edn, p.451. Blackwell Scientific Publications, Oxford.
- SILBERT, S., LIPPMANN, H.I. & GORDON, E. (1953) Mönckeberg's arteriosclerosis. *Journal of the American Medical Association*, **151**, 1176.
- SMITH, B.S., WILLIAMSON, N. & MCCONKEY, B. (1967) Dermal connective tissue in patients with chronic obstructive airways disease. *Lancet*, **i**, 341.
- SMITH, K.R. (1969) Electron microscopy of giant cell (temporal) arteritis. *Journal of Neurology, Neurosurgery and Psychiatry*, **32**, 348.
- VERHAGEN, A.R. & WOERDEMAN, M.J. (1975) Post-inflammatory elastolysis and cutis laxa. *British Journal of Dermatology*, **92**, 183.
- WILLIS, I., KLIGMAN, A. & EPSTEIN, J.H. (1973) Effects of long ultraviolet rays on human skin: Photoprotective or photoaugmentative? *Journal of Investigative Dermatology*, **59**, 416.

A new apparatus for the delivery of high intensity UVA and UVA+UVB irradiation, and some dermatological applications

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SUMMARY

A new apparatus consisting of a 200 W super pressure mercury lamp and a flexible light guide with a liquid light-conducting core is presented.

Its transmission for UVA irradiation is more than threefold as compared to conventional quartz fibre optic bundles. The light guide is capable of transmitting density levels exceeding 1 W/cm^2 . UVA and UVB are transmitted. By inserting a filter platelet (WG 345) into the light guide the UVB is cut off. The output of the unfiltered guide is 700 mW (3570 mW/cm^2), of the filtered guide 400 mW (2040 mW/cm^2), centered mainly at 366 nm.

The light apparatus proved useful for several dermatological applications. It replaces in many respects conventional Kromayer lamps, especially because the flexible light guide, which is 160 cm long and weighs only 210 g, can be brought into direct contact with the skin. Results of determining the minimal erythema dose (MED), of phototesting (8-MOP photoallergy, persistent light reactor, erythropoietic protoporphyria) and of photochemotherapy are given.

Among the artificial light sources which emit ultraviolet light and are being used in dermatology, the main ones are: (i) xenon arc; (ii) high and medium pressure mercury arcs; (iii) fluorescent tube lamps, belonging to the low pressure arcs with 'phosphors' on the glass envelope (Magnus, 1976a; Urbach, 1969).

The Kromayer lamp, a medium pressure mercury vapour arc, was especially useful for clinical purposes. It is a water-cooled lamp which has to be brought into direct contact with the skin when being used for light testing. Advantages of this light source were the relative ease of handling, short exposure times in testing procedures, and the ability to work with small circumscribed skin areas. The Kromayer lamp has a similar spectral emission to mercury vapour lamps, between 248 and 436 nm (Kromayer-quartzlamp, Hanau, Germany). Unfortunately, the Kromayer lamp of the original

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Hanau Quarzlampengesellschaft is not produced any more. Recently high intensity narrow-band lamps and preliminary studies of their irradiation effects on human skin were described (Alsins *et al.*, 1975). The following report introduces a new apparatus which replaces the Kromayer lamp and which has, in addition, several new features and applications.

Physical properties

The light source consists of a 200 W super pressure mercury lamp (Osram, HBO 200, Germany). The ultraviolet irradiation of the mercury lamp is transmitted and delivered by a new flexible light guide, which is not based on the usual concept of glass fibre optics, the UV irradiation being conducted by a liquid core. The optical coating material is made of a polymer having a lower refractive index than the light conducting liquid. The numerical aperture ($NA = \sqrt{n_1^2 - n_2^2}$) of this light guide is 0.49 (n_1 = refractive index of the core material, n_2 = refractive index of the coating material). Accordingly the light acceptance angle of the light guide is 60° . The diameter of the light conducting core is 5 mm, the total length is 160 cm, and the weight 210 g. With this new light apparatus, UVA + UVB or UVA alone (after insertion of an appropriate filter) can be delivered. Fig. 1 shows the

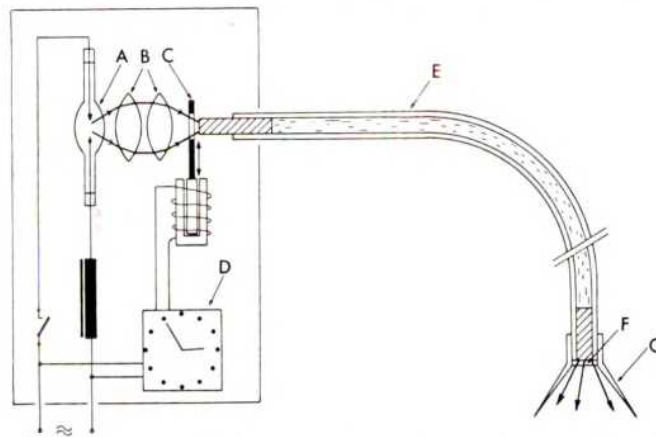


FIGURE 1. Apparatus for the delivery of high intensity UVA and UVA+UVB irradiation. The 160 cm long flexible light guide weighs only 210 g; a plastic cone is attached.

flexible delivery system for UVA+UVB and UVA irradiation respectively. The super pressure mercury lamp (A) is focused into the light entrance end of the flexible liquid light guide (E) by a quartz condensor (B) through an entrance slit (C). A removable filter platelet (F) (WG 345, Schott, Germany) at the light exit surface of the light guide transmits UVA irradiation ($320 \text{ nm} \leq \lambda \leq 400 \text{ nm}$) and absorbs UVB and UVC irradiation ($\lambda \leq 320 \text{ nm}$). The transmission in the spectral range of UVA ($\lambda = 366 \text{ nm}$) is three times higher than in known quartz fibre optic bundles having the same geometry, when both light guides are adapted to the same super pressure mercury lamp. A conically shaped plastic holder (plastic cone) (G) made of transparent acrylic glass serves to maintain a constant working distance. Accurate exposure times are possible by means of a timer (D). The unfiltered output at the light exit face of the light guide is 700 mW (3570 mW/cm^2), centered mainly at 366 nm . The output was measured by a calibrated bolometer (Coherent Radiation, Model 201, U.S.A.). The spectral distribution of the unfiltered output of the light guide is shown in Fig. 2. The spectral distribution was measured by a high intensity grating monochromator (Bausch and Lomb, U.S.A.), substituting the mercury arc lamp of the monochromator by the light exit face of our light guide, and a high sensitivity bolometer (Hewlett Packard, model 8330A, U.S.A.). As seen in Fig. 2, UVB and a

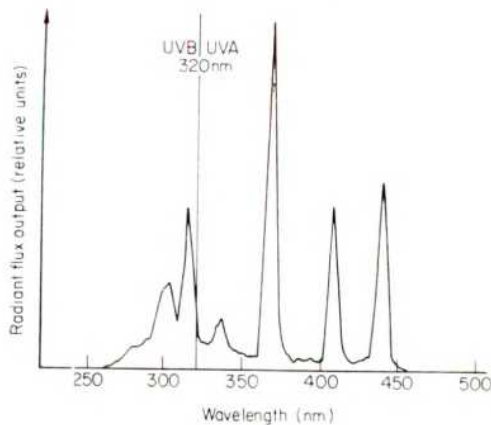


Fig 2

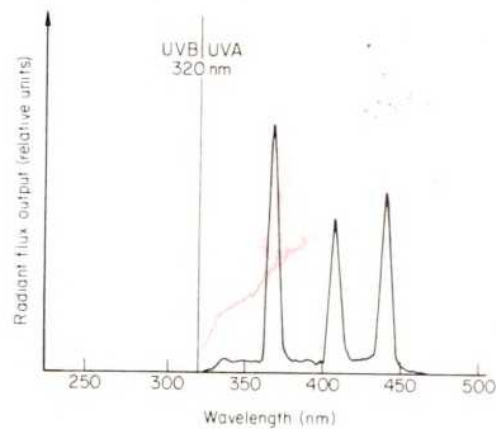


Fig 3

FIGURE 2. Wavelengths and radiant flux output, OSRAM HBO 200 super pressure mercury lamp. Unfiltered light guide emitting UVA + UVB.

FIGURE 3. Wavelengths and radiant flux output, OSRAM HBO 200 super pressure mercury lamp. Filtered light guide (WG 345) emitting UVA and visible light.

small amount of UVC irradiation ($\lambda \leq 280$ nm) are present in the total output. Fig. 3 shows the spectral distribution of the light guide with a WG 345 filter in place. UVB irradiation is practically eliminated. In this case the total output of the light guide decreases to 400 mW (2040 mW/cm²). About 25% of the intensity of the 366 nm line is lost by inserting this filter platelet.

Other light sources such as a 75 or 150 W xenon arc lamp* may be inserted instead of the super pressure mercury lamp (HBO 200).

Dosimetry

The intensity of the emitted light is given in mW/cm². We use a thermopile, model 17 and a watt-meter, indicator model 154 (Laser Instrumentation Ltd, England). Measurements were made daily for exact therapeutic doses and to recognize the decrease of intensity of the light source. For example, after 214 operating h, the intensity of the super pressure mercury lamp decreased by 29%.

Dermatological applications

Photobiology in dermatology includes phototherapy, photochemotherapy, and among others, phototesting. Examples of the last are reproduction of skin lesions *en miniature* in light-mediated hypersensitivities and demonstration of abnormal spectral activity. The usefulness of the new apparatus (Nath *et al.*, 1976) for the delivery of high intensity UVA and UVB irradiation will be described below.

Minimal erythema dose

For the determination of the minimal erythema dose (MED) wave lengths between 248 and 302 nm are necessary (Rottier, 1962), a spectral range of short and medium UV light. Between 250 and 260 nm and again at 297 and 302 nm the most erythematogenic effects can be expected (Magnus, 1976b).

There are several definitions of the MED. We adhere to the definition of Willis & Kligman (1970), who define the MED as 'the least light dose to give uniform redness with sharp margins'.

* Further inquiries about the physical properties of the light apparatus may be addressed directly to Dr rer. nat. Nath, Steinerstr. 15, 8 Munich 15, Germany.

The new light apparatus is well suited for MED testing. The high intensity of the unfiltered conductor (UVA + UVB) permits extremely short exposure times, in the range of a few seconds. Non sun-exposed sites, e.g. the buttocks, are chosen. Testing is possible either on the sitting or on the standing patient. For this reason the flexible light conductor proved to be superior to other conventional light sources such as the xenon lamp. The distal opening of the light conductor measures 0.5 cm in diameter when brought into direct contact with the skin and allows testing on small areas. The high intensity of the emitted light leads to heat development on the skin surface. Therefore wedge shaped, translucent plastic cones of various sizes are placed on the tip of the light conductor (Fig. 4a, b). Increasing the target-skin distance lessens undesirable heat development. Four cones are mainly used with the following specifications, in which the first figure stands for the length of the plastic cone and the second for the diameter of the irradiated skin: 0.5 cm/2.5 cm ϕ ; 1.0 cm/3.4 cm ϕ ; 1.5 cm/4.0 cm ϕ ; 2.0 cm/4.4 cm ϕ . We prefer a plastic cone of 4 cm diameter for MED testing (Fig. 5a). With this cone the MED in normal skin is elicited with between 2 and 18 s exposure time (0.07–0.60 J/cm²) depending on the skin type (type I–VI) (Protocol, 1975).

Reproduction of skin lesions and demonstration of abnormal spectral activity

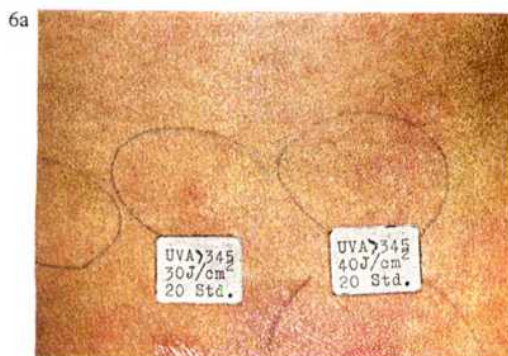
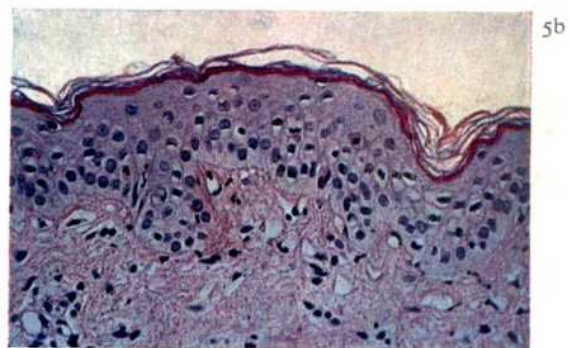
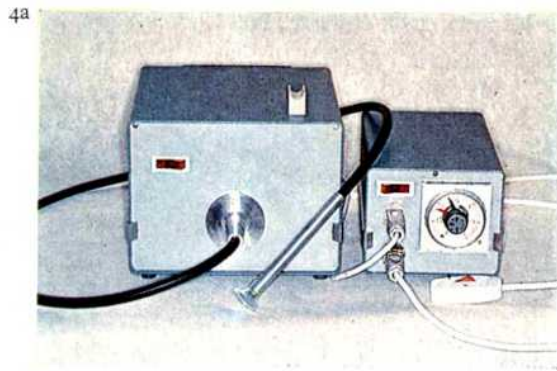
Phototoxic and photoallergic dermatitis to 8-methoxypsoralen (8-MOP). Drug-induced and light mediated hypersensitivity can develop from topical, oral or parenteral administration. Action spectra may correspond to the absorption spectra of the chemicals or may vary considerably. For instance the absorption spectrum for 8-MOP is at 217, 248 and 303 nm, whereas the action spectrum for the phototoxic reaction, which is used for instance in 8-MOP photochemotherapy of psoriasis, lies in the 330–360 nm range (Pathak, Krämer & Fitzpatrick, 1974). The new light apparatus turned out to be useful for many investigative and clinical studies following topical or oral 8-MOP application in man and in animals. For instance the minimal phototoxic dose (MPD) which is established in many cases prior to 8-MOP-UVA (PUVA) treatment (Protocol, 1975; Wolff *et al.*, 1975; Hofmann, Plewig & Braun-Falco, 1976 a,b) can easily be obtained with the filtered light conductor (UVA).

Contact photoallergy to 8-MOP is a rare phenomenon (Fulton & Willis, 1968; Sidi & Bourgeois-Gavardin, 1953; Ljunggren, 1977). Photoallergy to 8-MOP after oral intake has not been described before. We have observed a case of photoallergic dermatitis after either oral or topical application in a patient undergoing 8-MOP-UVA-treatment. This case was identified by means of the new light apparatus.

FIGURE 4. (a) Light apparatus (left) with flexible light conductor and timer (right). (b) Tip of the flexible light conductor with attached translucent plastic cone for irradiation of nails. For photochemotherapy the cone is in direct contact with the nail.

FIGURE 5. Minimal erythema dose (MED). (a) Determination of the MED with the unfiltered light conductor (UVA + UVB). Dose increments from top to bottom and left to right 0.04–1.0 J/cm² = 12–30 s. Test sites 14 h after irradiation on the buttocks in a well tanned subject, skin type IV (Protocol, 1975). The second site from below to the left is the MED (0.60 J/cm²). (b) Effects of UVA + UVB irradiation on human lower abdominal skin 24 h after application of a nine-fold MED. Skin type III. Dyskeratotic cells throughout the viable epidermis. Intra- and intercellular oedema. Disseminated lymphocytic infiltrate in the upper dermis (H & E, $\times 200$).

FIGURE 6. Photoallergy to 8-MOP. (a) Papulo-vesicular reaction in a 36-year-old female patient 20 h after ingestion of 40 mg 8-MOP, which was given 2 h prior to the application of 30 and 40 J/cm² with the filtered light conductor (UVA). (b) A biopsy from a positive test site of the same patient 16 h after phototesting. Pronounced lymphocytic and perivascular infiltrate in the upper and middle dermis with incipient spongiosis (H & E, $\times 40$).



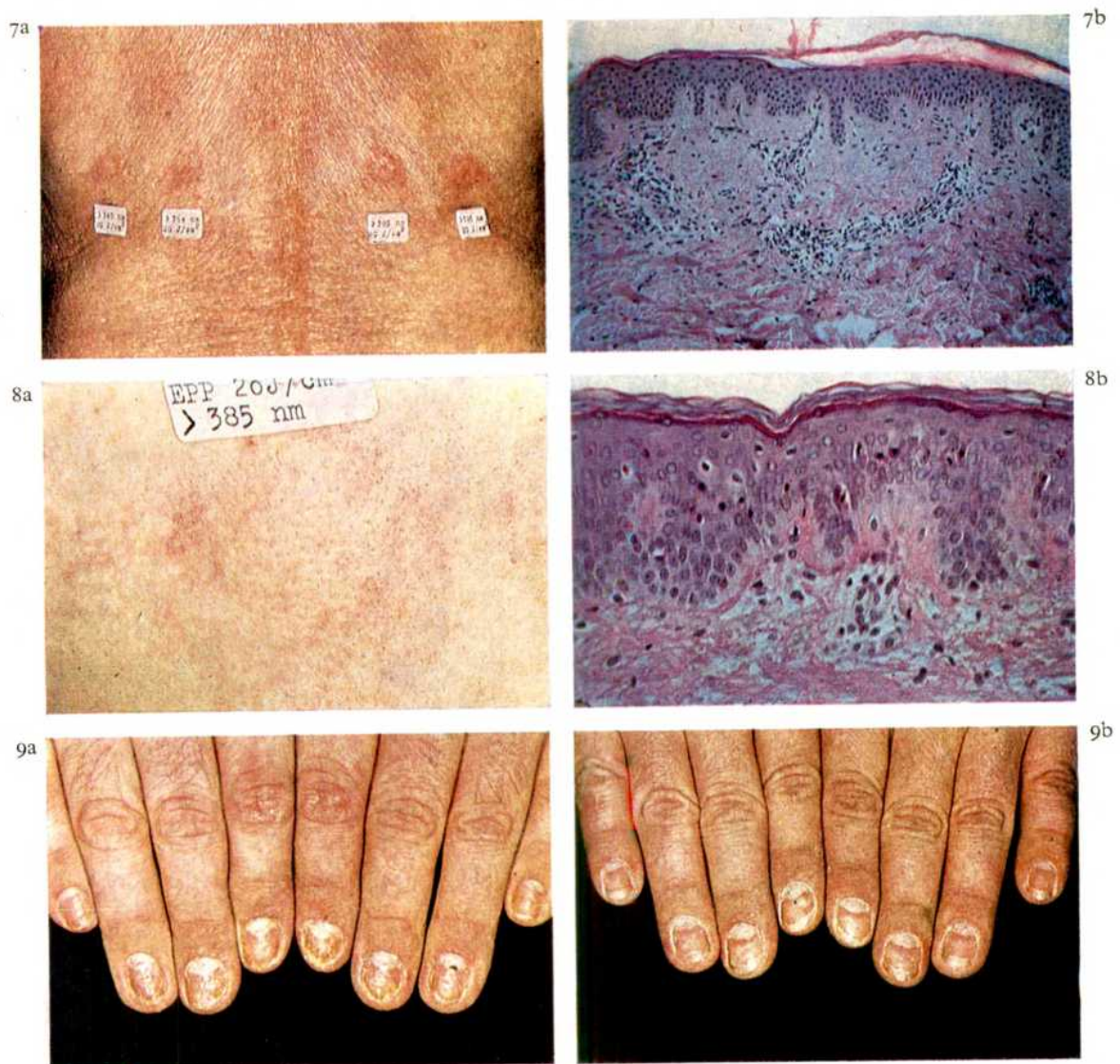


FIGURE 7. Persistent light reactor. Reproduction of skin lesions. (a) Erythematous urticarial lesions on the lower back 20 min after application of 20 J/cm² with the filtered light conductor (UVA) and one of the filters WG 345, 360, 385, or 395. The lesions persisted for many days and finally turned into indurated eczematous lesions. (b) Predominantly perivascular lymphocytic infiltrate and oedema in the upper and middle dermis 5 days after application of 20 J/cm² UVA > 360 nm (H & E, $\times 40$).

FIGURE 8. Erythropoietic protoporphyria. Reproduction of skin lesions. (a) Slightly erythematous and urticarial *peau-d'orange* lesion 20 min after application of 20 J/cm² UVA > 385 nm with the filtered light conductor (WG 385). (b) Diffuse oedema and sparse perivascular round cell infiltrate in the upper dermis. Deposits of hyalin throughout the upper dermis. Many dyskeratotic cells in the lower and middle stratum Malpighii 24 h after application of 15 J/cm² UVA at 570 $\pm$ 19.2 nm (high intensity grating monochromator).

FIGURE 9. Photochemotherapy of psoriasis of fingernails (a) Before treatment (28.10.1975). Typical psoriatic involvement of nail beds and nail plates. (b) After 43 treatments (20 weeks) with the filtered light conductor (UVA) (30.3.1976). 8-MOP was applied topically.

Case report. A 36-year-old female patient developed a widespread, itching, papulo-vesicular dermatitis after 16 uneventful PUVA treatments, 6 h after UVA irradiation and ingestion of 40 mg of 8-MOP (Meladinine, Basotherm, Germany). Oral challenge with the psoralen and 6.8–10.0 J/cm² reproduced the same clinical picture repeatedly. Topical application of a 0.15% 8-MOP solution (Meladinine) and application of 1.0, 3.0, 5.0 and 10.0 J/cm² UVA with the light apparatus 1 h later induced in less than 20 h a papulo-vesicular dermatitis of a crescendo type within all test sites. Two biopsies, one after the initial outbreak of photoallergy after PUVA treatment (Fig. 6a) and one from the experimental test site (Fig. 6b) revealed histological features of photoallergy (Plewig, Hofmann & Braun-Falco, 1977).

Persistent light reactor

The first report of a persistent light reactor (PLR) probably appeared as early as 1933 by Haxthausen. It is known that various chemicals such as halogenated salicylanilides play an important role in persistent light reactions (Jillson & Baugham, 1963; Epstein, Wuepper & Maibach, 1968). Action spectra in PLR vary considerably, and are reported for short and medium wave lengths or within the long UVA range (Freeman & Knox, 1968; Cripps & Enta, 1970). It is concluded from these results (Magnus, 1976) that without the influence of chemicals patients with PLR react to the erythematogenic medium wave lengths, and that skin exposed to chemicals such as halogenated salicylanilides reacts mainly in the long UVA range.

Case report. We studied a 73-year-old male patient with PLR who had positive photopatch tests to trichlorosalicylanilide and tribromosalicylanilide and a positive patch test to potassium dichromate, *p*-phenylenediamine and benzocaine. The filtered light conductor (UVA) and various edge filters (WG 345, 360, 385, 395, Schott, Germany) were used. The test area was the lower back. Each site received 20.0 J/cm² of UVA. Six h later an elevated urticarial erythema developed within all test sites and persisted for several days (Fig. 7a). This was followed by an eczema-like lesion 5 days later. A biopsy from such an eczematous lesion on day five showed a lymphocytic perivascular infiltrate in the upper dermis with incipient exocytosis into the epidermis (Fig. 7b).

Erythropoietic protoporphyria

Erythropoietic protoporphyria (EPP) is an uncommon disorder with its onset in childhood. Protoporphyrins are increased in the serum; these stem from erythrocytes and sensitize the skin against sun light. Patients suffer from burning and itching in the light-exposed skin shortly after exposure to the sun, often followed by erythema, oedema and urticarial swelling (Ippen, 1974; Schmidt *et al.*, 1974). Action spectra in EPP lie in the long UVA and in the visible range between 400 and 800 nm. Phototesting is, however, negative in 50% of the cases (Magnus, 1976c). This may possibly be due to insufficient light doses.

We studied three patients, two males and one female, with biochemically proven EPP (positive erythrocyte fluorescence, elevated serum protoporphyrins, etc.) with the new light apparatus. The light conductor was mounted with an edge filter (WG 385) and 15.0, 17.5 and 20.0 J/cm² applied. It was possible to reproduce lesions of EPP *en miniature* in all patients. For instance in a 21-year-old male patient 15.0 J/cm² of UVA > 385 nm induced an immediate erythematous reaction on the lower back, whereas 17.5 and 20.0 J/cm² caused an urticarial type skin reaction immediately after light exposure (Fig. 8a). Itching preceded the erythema and weals. The erythema was still visible after 24 h. In the second patient, a 33-year-old woman, positive reactions to UVA light > 385 nm appeared after 10 h and were still visible 14 h later. The time intervals of the experimentally induced symptoms in our cases of EPP coincided with the patients' clinical histories.

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Skin biopsies 24 h after phototesting revealed mainly dilatation of the blood vessels, and oedema in the upper dermis and papillary body. There were deposits of hyalin throughout the upper dermis (Fig. 8b).

Photochemotherapy, 8-MOP-UVA therapy

Photochemotherapy with *topical* administration of 8-MOP and UVA was introduced into medicine for the treatment of vitiligo by El Mofty (1948) and later proposed for the treatment of psoriasis vulgaris (Mortazawi, 1972). Parrish *et al.* (1974) and Wolff *et al.* (1975, 1976) reported very good results with the *oral* use of 8-MOP in photochemotherapy of psoriasis. In these reports exact therapeutic guidelines concerning drug and light dose were given, which in combination with high intensity treatment units made these results possible. Using their criteria, we have had technical experience with a variety of treatment units (Hofmann, Plewig & Braun-Falco, 1976a), and have reported the clinical and histological results in a larger series of patients (Hofmann, Plewig & Braun-Falco, 1976b; Braun-Falco, Hofmann & Plewig, 1977).

Photochemotherapy of psoriasis of the nails requires exceedingly high UVA intensity. Preliminary measurements show that about 90% of the UVA is absorbed by the nail plate and does not reach the nail bed (unpublished data). It was therefore necessary to look for a new light source with a higher UVA intensity than hitherto available. The new apparatus seems to fulfill these requirements. The light intensity is around 2040 mW/cm², 250 fold the intensity of modern treatment units for total body UVA irradiation, which for comparison have an output of around 8 mW/cm² (Parrish *et al.*, 1974; Wolff *et al.*, 1975, 1976; Hofmann, Plewig & Braun-Falco, 1976, a, b).

Photochemotherapy of psoriatic nails using the new light apparatus can be two-fold.

Topical application. A 0.15% 8-MOP solution (Meladinine, Basotherm, Germany) is painted, 2 and 1 h before UVA irradiation, onto the nail plate and nail fold, and foil-occluded.

Oral administration. 8-MOP is taken as capsules (Oxsoralen, Elder, U.S.A.) or tablets (Meladinine, Basotherm, Germany), depending on the body weight of the patient 2 h before UVA irradiation. For photochemotherapy of the nails with either topical or oral 8-MOP application, an initial UVA dose of 28 J/cm² was chosen (2 min of exposure time). The UVA dose was increased to a maximum of 140 J/cm² (10 min). No other medication was given during this period.

Twenty patients were treated in this way. Out of these, 11 had subungual psoriatic lesions, pits of the nail plate, and partial onycholysis; two patients had, in addition, subungual hyperkeratosis and onychodystrophy; three patients had acrodermatitis continua suppurativa (Hallopeau).

Topical application N = 31. In five patients good results or total clearing were achieved (Fig. 9a, b). There was no improvement in three patients. The mean number of treatments was 30, the mean duration of therapy 2.5 months. From these preliminary data we extrapolate that the results with topical application of 8-MOP for photochemotherapy in psoriatic nails are not impressive.

Acrodermatitis continua suppurativa (Hallopeau) N = 3

Two patients with this disabling disease were treated topically, one patient orally. After a mean number of 26 treatments, which approximates 2 months, good improvement was obtained.

Oral administration N = 4. Despite some good results in improving psoriatic nails following conventional oral 8-MOP-UVA therapy for total body therapy, this treatment was not sufficient in

many other cases successfully to treat the involved nails. In these patients we prefer an additional treatment of the nails with the light apparatus immediately following the total body UVA irradiation. After a mean treatment time of three months all four patients substantially improved, finally leading to normal regrowth of the nail plates.

Our preliminary experience suggests that for acrodermatitis continua suppurativa without nail destruction or inflammatory complications the topical administration of 8-MOP and UVA irradiation is sufficient. Cases with joint involvement should undergo oral 8-MOP administration.

Due to the relatively slow nail plate growth return to normal of diseased nails in psoriasis cannot be expected before 8–10 weeks of therapy. The rate of nail growth in psoriatics and healthy controls was established by Dawber in 1970. The mean growth rate of nails in psoriatics was 0.125 mm per day compared to under 0.10 mm in controls (Dawber, 1970).

ACKNOWLEDGMENTS

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REFERENCES

- ALSINS, J., CLAESSON, S., FISCHER, T. & JUHLIN, L. (1975) Development of high intensity narrow-band lamps and studies of the irradiation effect on human skin. *Acta dermato-venereologica*, **55**, 261.
- BRAUN-FALCO, O., HOFMANN, C. & PLEWIG, G. (1977) Feingewebliche Veränderungen unter Photochemotherapie der Psoriasis. Eine histologische und histochemische Studie. *Archives for Dermatological Research*, **257**, 307.
- CRIPPS, D.J. & ENTA, T. (1970) Absorption and action spectra studies on bithionol and halogenated salicylanilide photosensitivity. *British Journal of Dermatology*, **82**, 230.
- DAWBER, R. (1970) Fingernail growth in normal and psoriatic subjects. *British Journal of Dermatology*, **82**, 454.
- EL MOFTY, A.M. (1948) A preliminary report on the treatment of leucoderma with *Ammi majus* Linn. *Journal of the Royal Egyptian Medical Association*, **31**, 651.
- EPSTEIN, J.H., WUEPPER, K.D. & MAIBACH, H.I. (1968) Photocontact dermatitis to halogenated salicylanilides and related compounds. *Archives of Dermatology*, **97**, 236.
- FREEMAN, R.G. & KNOX, J.M. (1968) The action spectrum of photocontact dermatitis caused by halogenated salicylanilide and related compounds. *Archives of Dermatology*, **97**, 130.
- FULTON, J.E. & WILLIS, I. (1968) Photoallergy to methoxsalen. *Archives of Dermatology*, **98**, 445.
- HAXTHAUSEN, H. (1933) Persistent hypersensitivity to light after intravenous injections of tryptaflavine. *British Journal of Dermatology*, **45**, 16.
- HOFMANN, C., PLEWIG, G. & BRAUN-FALCO, O. (1976a) Technische Erfahrungen mit der 8-Methoxypsoralen-Photochemotherapie bei Psoriasis vulgaris. *Hautarzt*, **27**, 277.
- HOFMANN, C., PLEWIG, G. & BRAUN-FALCO, O. (1976b) Klinische Erfahrungen mit der 8-Methoxypsoralen-UVA-Therapie (Photochemotherapie) bei Psoriasis. *Hautarzt*, **27**, 588.
- IPPEN, H. & GOERZ, G. (1974) *Photodermatosen und Porphyrien*. H. Ippen, Düsseldorf.
- JILLSON, O.F. & BAUGHAM, D.D. (1963) Contact photodermatitis from bithionol. *Archives of Dermatology*, **88**, 409.
- KROMAYER-QUARZLAMPE. Technical features and instruction. Order No. 0501, Original Hanau Quarzlampe GmbH, D-6450 Hanau, Germany.
- LJUNGGREN, B. (1977) Psoralen photoallergy caused by plant contact. *Contact Dermatitis*, **3**, 85.
- MAGNUS, I.A. (1976) *Dermatological Photobiology. Clinical and experimental aspects*, (a) pp. 41–53; (b) pp. 117–146; (c) pp. 236–260. Blackwell Scientific Publications, Oxford.
- MORTAZAWI, S.A.M. (1972) Melanin and UVA bei Vitiligo, Psoriasis, Parapsoriasis und Acne vulgaris. *Dermatologische Monatschrift*, **158**, 908.
- NATH, G., PLEWIG, G., HOFMANN, C., KREITMAIR, A. & BRAUN-FALCO, O. (1976) A new apparatus for the delivery of high intensity UVA and UVB irradiation and some dermatological applications. Scientific Exhibit, VII. *International Congress on Photobiology, Rome 29.8. 1976–3.9. 1976*.

- PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, L. & PATHAK, M.A. (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. *New England Journal of Medicine*, **291**, 1207.
- PATHAK, M.A., KRÄMER, D.M. & FITZPATRICK, T.B. (1974) Photobiology and photochemistry of furocoumarins (psoralens). In: *Sunlight and Man* (ed. by T. B. Fitzpatrick, M. A. Pathak, L. C. Harber, M. Seiji and A. Kukita), pp. 335-368. University of Tokyo Press, Tokyo.
- PLEWIG, G., HOFMANN, C. & BRAUN-FALCO, O. (1977) Photoallergic dermatitis from 8-methoxypsoralen. *Archives for Dermatological Research*. Submitted for publication.
- Protocol (1975) European co-operative clinical trial, oral 8-methoxypsoralen photochemotherapy of psoriasis.
- ROTTIER, P.B. (1962) Untersuchungen mit einem kleinen Spektrographen zur Bestimmung der Hautempfindlichkeit gegenüber 100 und 250 mμ. *Strahlentherapie*, **119**, 591.
- SCHMIDT, H., SUITKER, G., THOMSEN, K. & LINTRUP, J. (1974) Erythropoietic protoporphyria. A clinical study based on 29 cases in 14 families. *Archives of Dermatology*, **110**, 58.
- SIDI, E., BOURGEOIS-GAVARDIN, J. (1953) Mise au point du traitement du vitiligo par 'l' Ammi Majus'. *Presse médicale*, **61**, 436.
- URBACH, F. (1969) Solar simulation for phototesting of human skin. In: *The Biologic Effects of Ultraviolet Radiation* (ed. by F. Urbach), pp. 107-114. Pergamon Press, Oxford.
- WILLIS, I. & KLIGMAN, A.M. (1970) Aminobenzoic acid and its esters. The quest for more effective sunscreens. *Archives of Dermatology*, **102**, 405.
- WOLFF, K., HÖNIGSMANN, H., GSCHNIT, F. & KONRAD, K. (1975) Photochemotherapie bei Psoriasis. Klinische Erfahrungen bei 152 Patienten. *Deutsche medizinische Wochenschrift*, **100**, 2471.
- WOLFF, K., FITZPATRICK, T.B., PARRISH, J.A., GSCHNIT, F., GILCHREST, B., HÖNIGSMANN, H., PATHAK, M.A. & TANENBAUM, L. (1976) Photochemotherapy for psoriasis with orally administered methoxsalen. *Archives of Dermatology*, **112**, 943.

Oral psoralen photochemotherapy of atopic eczema

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SUMMARY

Oral administration of 8-methoxypsoralen followed by exposure to a high-intensity longwave ultraviolet treatment system resulted in clearing of atopic eczema in 15 patients. Paired-comparison studies demonstrated that this treatment was superior both to conventional ultraviolet light therapy and to no treatment. Short-term observation suggests that regular therapy is necessary to maintain remission of the disease.

Atopic eczema is a common disorder which usually first appears in childhood, runs a variable course over months to years, and frequently persists into adult life. The condition is genetically determined and is one manifestation of the atopic diathesis which includes a tendency to form reaginic antibodies and to have symptomatic asthma, hay fever, and eczema. The cardinal symptom of atopic eczema is pruritus and this gives rise to the clinical features of widespread excoriations and lichenification. The disfigurement and intractable pruritus may result in a considerable disturbance of the patient's physical, social, and psychological well-being.

Atopic eczema is also a source of chronic frustration for the physician. Topical and, to a much lesser extent, oral corticosteroids are currently the mainstay of treatment in all but the mildest cases. Even when used in large doses, these usually provide only partial symptomatic relief. Apart from the morbidity of the condition itself, the treatment also results in a significant morbidity. Topical corticosteroids can cause atrophy and telangiectasia of the skin while oral steroids, as well as the prolonged use of topical steroids on diseased skin, can result in systemic side effects.

It is a clinical observation that some patients with atopic eczema are often better during the summer months. This led to the present study of the value of phototherapy. Oral psoralen photochemotherapy using sunlight has been widely used in vitiligo for many years and more recently oral methoxypsoralen in combination with a high-density longwave ultraviolet light system (GTE Sylvania) have been shown to control psoriasis effectively (Parrish *et al.*, 1974) and to be beneficial in some patients with vitiligo (Parrish *et al.*, 1976) and mycosis fungoides (Gilchrest *et al.*, 1976). The treatment involves the ingestion of psoralens (P) followed, at a specified interval, by exposure to longwave ultraviolet radiation (UV-A), abbreviated PUVA. The present study was designed to evaluate PUVA therapy of atopic eczema and to compare PUVA with conventional ultraviolet irradiation (UV-B).

PATIENTS AND METHODS

Fifteen Caucasians with severe atopic eczema uncontrolled by conventional topical and oral corticosteroid therapy were treated. The age range of the group was 9-63 years, with a mean of 27 years. All had a personal and/or family history of atopy and at the time of evaluation had a clinical picture consistent with the diagnosis of severe atopic eczema. The clinical features are listed in Table 1.

TABLE 1. Clinical picture. Area of body involved: 30-100% (mean: 80%)

Lesions	
Acute and sub-acute eczema	15
Chronic lichenification	6
Secondary infection	4
Nail dystrophy	3
Scalp involvement	4
Chronic blepharitis	4

Design of the study

The patients were divided into three groups each of five patients.

Group 1. Whole-body PUVA therapy. One site, 4 × 4 cm on the buttock, was shielded with an aluminum plate during UV-A exposures and served as a control area in four of the patients.

Group 2. PUVA versus no treatment. In a bilateral comparison, PUVA was used on one side of the body and no treatment apart from lubricants was given to the other side. The untreated side was shielded with two layers of cotton sheeting during irradiation.

Group 3. PUVA versus UV-B. In a bilateral comparison, PUVA was used on one side of the body and UV-B treatment was given to the other side. UV-B treatments were always given prior to psoralen ingestion.

In Group 1, the control site was kept covered during the treatments until 99% of the other areas had been cleared of eczema, and then the control site was treated and cleared. In Groups 2 and 3, the bilateral comparison was continued until 95% (rule of 9 for large areas; palm of hand = 1%, for small areas) of the PUVA-treated side had been cleared of eczema and then the other side was cleared with PUVA therapy.

Other medications

Patients were permitted unrestricted use of emollients and Atarax (hydroxyzine hydrochloride). Four of the five patients receiving whole-body PUVA therapy had their previous therapy of oral and topical steroids tapered off during the early phase of PUVA treatment to avoid the problem of a rebound effect. All corticosteroids were stopped prior to the study on the other patients so that none of the patients in the bilateral comparison study used any topical or oral steroids.

Laboratory investigations

The serum bilirubin, serum glutamic oxalacetic transaminase, lactic acid dehydrogenase, alkaline

phosphatase, blood urea nitrogen, creatinine, and IgE were measured, and a complete blood count, urinalysis, and culture of skin bacterial flora were performed prior to commencing therapy and were monitored during the study.

PUVA treatment

Oral 8-methoxypsoralen (0.6 mg/kg body weight) was ingested 2 h before exposure to UV-A radiation. The initial dose of UV-A (range 1.5–3.5 J/cm²) was determined by the patient's sunburn and suntan history, and this dose was increased by 0.5–1.0 J/cm² each treatment provided there was no significant erythema present. Treatments were given three times a week during the clearance phase with a minimum of 48 h between each treatment because a PUVA-induced erythema usually does not peak until at least 48 h. Once a patient was judged to be at least 99% free of clinically apparent disease and pruritus, frequency of treatments was reduced to weekly for 4–6 treatments. If during that time there was no exacerbation of eczema, the frequency was again reduced to one treatment every other week. Whole-body irradiation was provided by either a bed unit or an upright unit. The characteristics of these systems have been described previously (Parrish *et al.*, 1974; Levin & Parrish, 1974).

UV-B treatment

The initial dose of UV-B radiation (range 30–180 mJ/cm² of 275–320 nm radiation) was also determined by the patient's sunburn and suntan history. This dose was increased by 10–25% each treatment with the aim of maintaining a trace erythema. When a symptomatic erythema developed, depending on the degree, either the UV-B dose was held constant or treatment was withheld until the erythema had subsided. Treatments were given five times each week with a minimum of 24 h between each treatment. The characteristics of the UV-B irradiator have been described (Parrish *et al.*, 1976).

RESULTS

The response to whole-body PUVA therapy, PUVA compared to no treatment, and PUVA compared to UV-B, are summarized in Table 2. In the patients in Group 1, eczema was cleared from 99% of

TABLE 2. Treatment response

Group 1	
Whole-body PUVA	Eczema 99% clear in 20–58 treatments
Control site	Increased eczema in all patients
Group 2	
PUVA side	Eczema 95% clear in 12–21 treatments
Untreated side	No change
Group 3	
PUVA side	Eczema 95% clear in 7–12 treatments
UV-B side	Eczema increased in 4, no change in 1, in 13–19 treatments

the body surface in 20–58 PUVA treatments while the covered, control sites developed more severe eczema. In Group 2 patients, the PUVA-treated side was cleared to <5% eczema in 12–21 treatments and at that time the eczema on the untreated side was unchanged in all patients. At the end of the bilateral comparison, pruritus was present only on the untreated side. In Group 3 patients, the PUVA-treated side was cleared to <5% eczema in 7–12 treatments. Over the same period, 13–19 UV-B

treatments were given to the other side. On the UV-B-treated side the eczema had increased in severity and extent in 4 patients and was unchanged in the other patient. Clearance of eczema to $<1\%$ in all fifteen patients required 18–59 treatments (mean: 36 treatments) over 50–202 days (mean: 101 days) and a final clearance dose of 7–24 J/cm² of UV-A (mean: 13 J/cm²). The first sign of improvement was a decrease of the pruritus in 10 patients and a decrease in the extent and severity of the eczema in 5 patients. Improvement was noticeable after 4–21 treatments and thereafter was slowly progressive. At the time of 99% clearance, all patients were almost entirely free of pruritus. There was no apparent correlation in this study between the type of eczema and the response to treatment.

The maintenance treatment requirements varied. Eleven patients required continuation of weekly treatments, three were controlled by one treatment every second week, and the other patient stopped treatment of her own accord and after 3 months was still free of eczema having no treatment at all. During the follow-up maintenance phase of 4–16 months (mean: 9 months), seven patients had an exacerbation of their eczema involving more than 5% of body surface. In three patients this exacerbation was severe and involved $>60\%$ of the body surface. In six patients these flare-ups followed a reduction in the frequency of treatment during the stepwise procedure of finding the correct frequency for maintenance in the individual patient. One patient flared-up during the autumn and gave a history of autumnal exacerbations over the previous 4 years. The best indicator of a developing exacerbation was a return of pruritus, and this usually preceded the appearance of eczema by a week or more. All exacerbations responded to a return to two or three treatments per week as used in the clearance phase plus, in five patients, an increase in the dose of UV-A.

A marked exacerbation of eczema occurred in all Group 2 and 3 patients as their topical and oral steroid therapy was tapered prior to commencing PUVA therapy. In three patients the exacerbation of eczema was major and required hospitalization in two cases. Group 1 patients, who were gradually tapered off steroid therapy as their eczema came under control with PUVA, did not have any post-steroid exacerbations.

During the therapy, nail dystrophy cleared in the three patients in whom the nails were affected although this took several months. The scalp cleared in three of the four patients with scalp involvement without any specific treatment. After careful consideration and a detailed explanation to the patients, each patient with symptomatic blepharitis was given a short course of treatment (4–10 exposures) without goggles but with their eyes closed during the whole of the treatment. This was successful in clearing the blepharitis in three of the four patients affected and those patients have remained clear even though all have resumed wearing goggles.

Four patients complained of nausea, two only when the psoralen was ingested without food, and the other two only occasionally and this was unrelated to food ingestion. Six patients had a localized symptomatic erythema on PUVA-treated areas early in the course of treatment. Four of the five patients treated with UV-B also had a symptomatic localized erythema. One patient showed post-inflammatory depigmentation at the time of initial evaluation, while in three others loss of normal pigmentation was concealed by overlying lichenification and only became evident as therapy progressed. Because such depigmented areas readily developed phototoxicity, UV-A dosimetry had to be extremely accurate in these patients. In addition, three patients developed subungual haemorrhages of the fingernails, also presumably due to phototoxicity and this required wearing an opaque nail polish until the nails recovered.

During this study, recurrent herpes labialis was noted in three patients who gave a past history of the infection. In addition, herpes infection of the face with ocular involvement occurred in two patients, one of whom had no past history of herpes. These patients fully recovered with idoxuridine therapy locally and resumed PUVA treatments.

No abnormalities of the serum bilirubin, serum glutamic oxaloacetic transaminase, lactic acid de-

hydrogenase, alkaline phosphatase, blood urea nitrogen and creatinine assays, the complete blood count and urinalysis, developed during the treatment in any of the patients. Serum IgE levels and qualitative cultures of bacterial skin flora showed no significant change either during the bilateral comparison studies or over the course of clearing and maintenance treatment.

DISCUSSION

This study has shown in 15 patients that PUVA therapy is an effective clearance treatment for atopic eczema and that once the eczematous state has been brought under control it is possible to maintain a clear state with continued maintenance treatment. Furthermore, bilateral comparison studies showed that treatment with PUVA was clearly superior to UV-B therapy. It is interesting to note that all four patients who showed a worsening of their eczema on the UV-B-treated side gave a history of improvement during the summer months. Factors other than solar UV-B radiation (e.g. salt water, absence of stress, other portions of the solar electromagnetic spectrum) may be responsible for this improvement. Patients in these bilateral comparison studies did not receive any topical corticosteroids during the course of treatment. Lubricants alone were permitted as topical therapy.

The response to PUVA therapy in atopic eczema patients differs from that seen in psoriatic patients in several important respects. The number of treatments required to achieve signs of improvement and the number required to reach a cleared state are about twice that required in treating psoriasis (Parrish, 1976). In addition, a much more aggressive approach to maintenance therapy must be adopted. In psoriatic patients, a decrease in the frequency of maintenance treatment below the level necessary to control the disease may be followed by a slow return of the disease. In atopic eczema patients, the disease seems to recur rapidly if adequate maintenance is not given. Therefore, the eczema patients must strictly adhere to the maintenance schedule and they also must be carefully monitored for the return of pruritus, the first sign of a developing exacerbation. The long-term maintenance requirements of PUVA therapy in atopic eczema are not known. Finally, eczema patients differed from psoriatic patients psychologically, being more anxious, questioning and introverted. As a result they require much more support and, hence, more physician time than do psoriatic patients undergoing similar PUVA therapy.

The occurrence of herpes simplex infection during therapy is a worrisome observation. An increased frequency of herpes labialis is noted in psoriatic patients receiving PUVA and UV-B treatments (Morison, personal observation). Patients with atopic eczema are prone to widespread herpes simplex infection and the addition of a therapy known to precipitate such infections may create a serious risk in some patients. However, since both cases of widespread herpes simplex infection occurred in patients while they had extensive eczema it is not possible to decide whether this risk exists in patients who are clear and on maintenance treatment. Vigilance on the part of the physician and education of the patient are essential for early diagnosis and adequate management of herpes simplex.

The long-term effects of PUVA therapy are still being evaluated and will probably not be fully known for years. The two principal areas of concern are skin carcinogenesis and cataracts, both of which have been shown to occur in animals (Griffin, 1959; Logan, 1961), but as yet have not been reported in humans undergoing treatment. Patients in the present study had initial eye examinations and a yearly follow-up is planned.

The mechanism by which PUVA therapy induces an improvement in skin disease is not known. Inhibition of DNA synthesis may benefit the hyperproliferative state of psoriasis (Parrish *et al.*, 1974) and a cytotoxic effect on abnormal dermal cells may be the therapeutic mechanism in mycosis fungoides (Gilchrest *et al.*, 1976). However, in atopic eczema there is no increase in epidermal turnover and the inflammatory infiltrate in the dermis does not contain abnormal cells. The pathogenesis of atopic

eczema is largely unknown but an immunological disturbance has been suggested by many studies, and such a disturbance is possibly mediated by cells in the dermis. PUVA may well interfere with the function and viability of such cells and thus suppress the disease state. The observation in this study that eczema not exposed to PUVA did not clear indicates that the effect of PUVA is local and not systemic. However, until more is known about the action of PUVA and the aetiology of the disease, any suggestion as to the mechanism of the effect observed in the present study must remain conjectural.

This study is preliminary and provides only a short follow-up of the maintenance phase. A full evaluation of the role of PUVA therapy in the treatment of atopic eczema will require a larger study and a longer follow-up. However, at this point it appears to offer promise as a therapy for a condition which can be physically and psychologically crippling and for which there is no safe or effective treatment.

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REFERENCES

- GILCHREST, B.A., PARRISH, J.A., TANENBAUM, L., HAYNES, H.A. & FITZPATRICK, T.B. (1976) Oral methoxsalen photochemotherapy of mycosis fungoides. *Cancer*, **38**, 683.
- GRIFFIN, A.C. (1959) Methoxsalen in ultraviolet carcinogenesis in the mouse. *Journal of Investigative Dermatology*, **32**, 367.
- LEVIN, R.E. & PARRISH, J.A. (1974) Phototherapy of vitiligo. *Lighting Design and Application*, **4**, 38.
- LOGAN, D. (1961) Photosensitization and cataracts. *Archives of Ophthalmology*, **66**, 28.
- PARRISH, J.A., FITZPATRICK, T.B., SHEA, C. & PATHAK, M.A. (1976) Photochemotherapy of vitiligo with oral psoralen and a new high-intensity longwave ultraviolet light (UV-A) system. *Archives of Dermatology*, **112**, 1531.
- PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, L. & PATHAK, M.A. (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. *New England Journal of Medicine*, **291**, 1207.

The effects of photosensitizing antibiotics and ultraviolet irradiation on the biosynthesis of prostaglandins

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SUMMARY

Oxygenation of arachidonic acid *in vitro* by calf skin microsomal acetone powder was enhanced by UV-irradiation at wavelengths of 254 and 360 nm. Further enhancement of the oxygenation reaction was observed in the presence of two photosensitizing cyclic antibiotics, tetracycline and demethylchlortetracycline.

To test whether or not the oxygenation of arachidonic acid was related to the biosynthesis of prostaglandins, [$1-^{14}\text{C}$]-arachidonic acid was incubated with calf skin acetone powder in the presence of UV-irradiation and the cyclic antibiotics. Prostaglandin biosynthesis from arachidonic acid by the calf skin microsomal acetone powder was enhanced after exposure to UV-irradiation at 254 nm and moderately at 360 nm. Incubation in the presence of demethylchlortetracycline (0.2 mM) increased prostaglandin biosynthesis approximately 95% over control by UV-irradiation at 254 nm. No significant stimulation of prostaglandin biosynthesis was observed at 360 nm. Non-photosensitizing antibiotics had no effect either on the oxygenation of arachidonic acid or on the biosynthesis of prostaglandin with or without UV-irradiation.

Since a previous report from our laboratory had demonstrated that other photosensitizers also stimulate prostaglandin biosynthesis *in vitro* after exposure to UV-irradiation and since these photosensitizing cyclic antibiotics have been associated with cutaneous inflammatory reactions such as erythema, it is reasonable to suggest from these data that the inflammatory reactions associated with these photoreactive antibiotics may in part, be via the biosynthesis and release of the prostaglandins which are known to produce cutaneous inflammatory reactions.

Many studies have demonstrated that the skin has the ability to synthesize and metabolize prostaglandins (Ziboh & Hsia, 1971; Van Dorp, 1971; Jonsson & Angaard, 1972; Greaves & McDonald,

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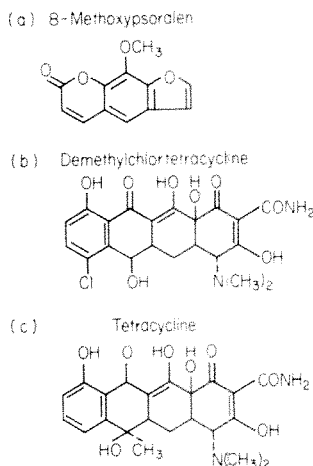


FIGURE 1. Chemical structures of some photoreactive substances. (a) 8-Methoxypsoralen (8-MOP); (b) Demethylchlortetracycline (Demeclocycline); (c) Tetracycline.

1972; Ziboh, 1973). Prostaglandin-like substances have been reported from areas of delayed inflammation in skin after exposure to ultraviolet irradiation (Mathur & Gandhi, 1972; Greaves & Sondergaard, 1970). More recently, exposure of human skin to UV-irradiation of 3 M.E.D. at 250 nm (UVC) resulted in the formation of prostaglandins (Plummer *et al.*, 1977). Treatment of skin with topical indomethacin after UV-irradiation (UVB) has also been reported to result in the suppression of erythema (Snyder & Eaglstein, 1974). These results suggest that a relationship exists between UV-irradiation, the formation and release of prostaglandins and the development of erythema in the skin.

In our continuing effort to elucidate the mechanism of action of photosensitizing compounds, we recently reported that a photosensitizing compound, 8-methoxypsoralen when exposed to UV-irradiation, markedly stimulated the formation of prostaglandins from arachidonic acid *in vitro* (Lord *et al.*, 1976). Among another group of compounds reported to cause blistering erythema are two cyclic antibiotics, tetracycline and demethylchlortetracycline (Falk, 1960; Cullen, Catalano & Helfman, 1966; Blank, Cullen & Catalano, 1968; Frost, Weinstein & Gomez, 1971; Schorr & Monash, 1963). The clinical manifestation common to these substances is the development of erythema after acute exposure to UV-irradiation. In view of some similarities in the structures of these two antibiotics and 8-methoxypsoralen (Fig. 1), it was of interest to ascertain whether or not these antibiotics are associated with the formation of prostaglandins because of their pro-inflammatory effects. In these experiments, we describe the ability of these photosensitizing compounds after exposure to ultraviolet irradiation to affect the biosynthesis of prostaglandins *in vitro*.

MATERIALS AND METHODS

[1-¹⁴C]-Arachidonic acid (specific radiopurity, 58.0 mCi/mmol) was purchased from New England Nuclear, Boston, Mass., U.S.A. Radiopurity was ascertained by TLC on silica gel G in solvent system chloroform-methanol-acetic acid (90:5:5, by vol). PGE₂, PGD₂, and PGF_{2α} were gifts from Dr U.F. Axen of the Upjohn Co., Kalamazoo, Michigan, U.S.A. Non-radioactive arachidonic acid was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. and purity was verified by TLC and gas liquid chromatography as reported previously (Ziboh, 1973). Purity was approximately 98%. Demethylchlortetracycline (declomycin) was a gift from the Lederle Co., Chicago, Ill., U.S.A. The

tetracycline and clindamycin used in these experiments were products of the Upjohn Co., Kalamazoo, Michigan, U.S.A. Sulfacytine and chloramphenicol were gifts from Parke-Davis Co., Detroit, Michigan, U.S.A. UV-irradiation sources used were obtained from a chromato-VUE light box, model CL20 with one major emission spectrum at 254 nm with an energy source of 1.4×10^{-3} J/cm²/s at 10 cm and another major emission spectrum at 360 nm with an energy source of 1.4×10^{-2} J/cm²/s at 10 cm, respectively.

Oxygenation of arachidonic acid by acetone powder from fetal calf skin

The oxidative cyclization of arachidonic acid to form prostaglandins involves a series of reactions. In order to measure the activity of the cyclo-oxygenase, the enzyme which catalyzes the first and rate limiting step of prostaglandin biosynthesis from precursor arachidonic acid (Smith & Lands, 1971), acetone powder of fetal calf skin microsomal fraction was prepared according to the procedure described by Wallach and Daniels (1971). The enzyme used in these studies was prepared by homogenizing 100 mg of the acetone powder in 1 ml of 0.1 M Tris-HCl (pH 8.5) in a small Dounce ball type homogenizer, 0.04 ml aliquot (4.0 mg) being used for each incubation. Similarly, the arachidonic acid (20:4, ω 6) used was prepared by suspending 50 mg in 1.0 ml of 0.1 M Tris-HCl buffer, pH 8.5 and 0.04 ml aliquot (2.0 mg) of this suspension was used for each incubation.

Various concentrations of the test substances previously sonicated into the 0.1 M Tris HCl were added into the incubation chamber before the initiation of the reaction. For incubations involving UV-irradiation the reaction chamber containing the enzyme alone or enzyme plus the test substances and substrate arachidonic acid was placed 10 cm directly under the UV-lamp and incubation carried out for 10 min. In other experiments the compounds were pre-irradiated for 10 min before incubation. The oxidation of arachidonic acid by the fetal calf skin microsomal cyclo-oxygenase was determined by measuring oxygen absorption at a constant temperature of $30 \pm 0.5^\circ\text{C}$ according to the procedure of Smith & Lands (1971). The total volume of each reaction mixture was 3.0 ml. Oxygen uptake was measured on a Yellow Springs Instrument Co., Model 53 oxygen monitor equipped with a model 5301 bath assembly as reported previously (Lord *et al.*, 1976). The monitor was attached to a Corning 800 strip recorder.

Biosynthesis of prostaglandins from ^{14}C -arachidonic acid

In order to ascertain whether or not the oxygenation of arachidonic acid was synonymous with the formation of the primary prostaglandins, ^{14}C -arachidonic acid was incubated with fetal calf skin acetone powder in the presence or absence of UV-irradiation. Briefly, [$1\text{-}^{14}\text{C}$]-arachidonic acid (0.2 μCi , 3.4 nmol) was incubated at 37°C for 10 min in a 5 ml medium containing fetal calf skin acetone powder (10 mg) and hydroquinone (0.55 mM) with no cofactors. Control experiments contained no enzyme or boiled enzyme preparations. Various concentrations of test substances suspended in 0.1 M Tris-HCl were added to the incubation mixture when needed. Exposure to UV-irradiation was similar to that described above. Fractionation and identification of radioactive prostaglandins by thin layer chromatography on silica gel G were as reported previously (Lord *et al.*, 1976).

RESULTS

Effects of antibiotics on the activity of the cyclo-oxygenase and biosynthesis of prostaglandins from arachidonic acid by fetal calf skin

In preliminary experiments, several concentrations of antibiotics were tested and 0.2 mM was found as a most effective dose for these studies. The effects of demethylchlortetracycline and tetracycline alone on the oxygenation of arachidonic acid and the conversion of [$1\text{-}^{14}\text{C}$]-arachidonic acid into

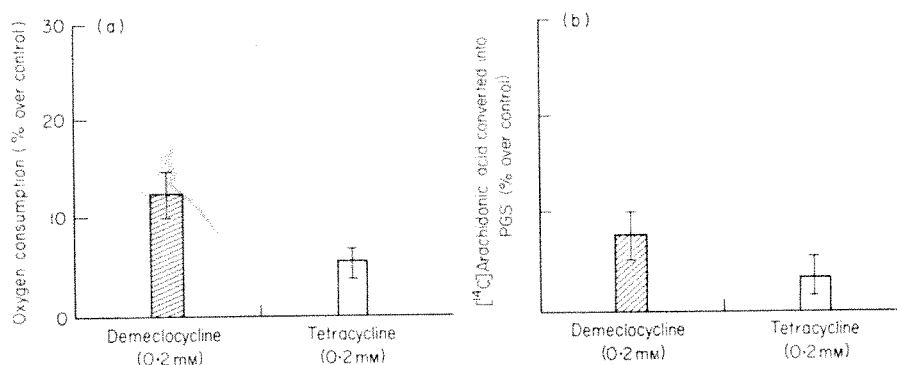


FIGURE 2. Effects of antibiotics on (a) the activity of the cyclo-oxygenase and (b) biosynthesis of prostaglandins from arachidonic acid.

(a) An aliquot of acetone powder of microsomal fraction from fetal calf skin (10 mg) previously suspended in 0.1 M Tris-HCl buffer, pH 8.5 was added to the incubation chamber containing 0.2 mM demeclocycline or tetracycline. After the addition of test substances, arachidonic acid (2.2 mM final concentration) was added to the incubation chamber to initiate the reaction. The reaction rate ($\mu\text{M O}_2/\text{min}$) was determined from continuous measurements of oxygen uptake with the oxygen electrode. Control experiments had no test substances added. Hatched column, ARA + DMC + ENZ; open column, ARA + TC + ENZ.

(b) An aliquot of acetone powder (10 mg) suspended in 0.1 M Tris-HCl buffer as indicated above was added into incubation vial containing sonicated test substances and 0.5 mM hydroquinone. After the addition of [$1\text{-}^{14}\text{C}$]-arachidonic acid (0.2 μCi) the mixture was incubated at 37°C for 10 min. One set of control experiments contained either no enzyme or boiled enzyme preparations and the other control experiment contained enzyme but no test substances. Hatched column, [^{14}C]ARA + DMC + ENZ; open column, [^{14}C]ARA + TC + ENZ.

The data expressed as percent over control are from the mean of duplicate determinations from 3 experiments. The vertical bars represent ranges and control is represented as 0.

prostaglandin are shown in Fig. 2 (a and b). Both antibiotics alone exerted very minor stimulatory effect on the activity of the cyclo-oxygenase and the formation of prostaglandins. For instance, oxygen consumption and prostaglandin biosynthesis from arachidonic acid by demeclocycline, the more potent of the antibiotics, were 12% and 8% respectively over controls.

Effects of antibiotics and UV-irradiation on the oxygenation of arachidonic acid by fetal calf skin acetone powder

Incubation of antibiotics, acetone powder and arachidonic acid directly under UV-irradiation at wavelengths of 254 and 360 nm are shown in Fig. 3 (a and b). Demeclocycline after irradiation at 254 nm (with an energy source of $1.4 \times 10^{-3} \text{ J/cm}^2/\text{s}$) enhanced oxygenation of arachidonic acid approximately 112% over controls whereas UV-irradiation alone in the absence of test substances enhanced oxidation approximately 40% (3a). Stimulation is at least 70% above the effect of UV-irradiation alone. In a similar experiment, the effect of tetracycline on the oxygenation reaction was approximately 58%, only 18% above the effect of UV-irradiation alone. Similar experiments at 360 nm (with a higher energy source of $1.4 \times 10^{-2} \text{ J/cm}^2/\text{s}$) resulted in approximately half the effect observed at 254 nm (with a lower energy source). In another experiment, the antibiotics were first exposed to UV-irradiation at both wavelengths for 10 min before the incubation of the irradiated antibiotics with arachidonic acid and acetone powder. Slightly but not significantly higher enhancement of the oxygenation of arachidonic acid was observed. These latter data suggest that the photoreactive products of the antibiotics are potent activators of the oxygenation of arachidonic acid.

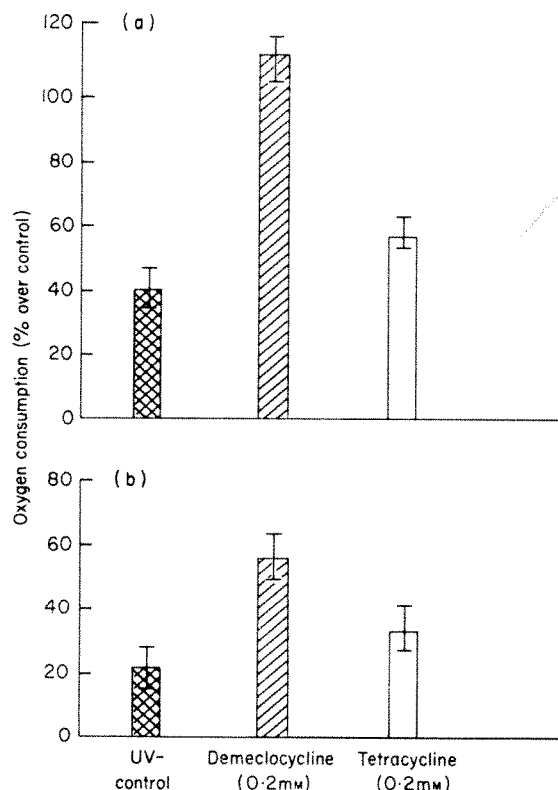


FIGURE 3. Effects of antibiotics and UV-irradiation on the oxygenation of arachidonic acid by fetal calf skin acetone powder.

The incubation conditions containing test substances were as described in the legend for Fig. 2(a). Arachidonic acid (2.2 mM) was added under UV-irradiation at 254 nm and 360 nm. The reaction rate ($\mu\text{M O}_2/\text{min}$) was determined from continuous measurements of oxygen uptake with an oxygen electrode.

(a) Data on the effects of antibiotics on the oxygenation reaction at 254 nm. Cross-hatched column, ARA + UV - 254 + ENZ; hatched column, ARA + DMC + UV - 254 + ENZ; open column, ARA + TC + UV - 254 + ENZ.

(b) Data on the effects of antibiotics at 360 nm. Cross-hatched column, ARA + UV - 360 + ENZ; hatched column, ARA + DMC + UV - 360 + ENZ; open column, ARA + TC + UV - 360 + ENZ.

The data expressed as per cent over control are from the mean of duplicate determinations from 3 experiments. The vertical bars represent ranges.

Effect of antibiotics and UV-irradiation (254 nm) on the biosynthesis of prostaglandins from ^{14}C -arachidonic acid

Since oxygenation of arachidonic acid by fetal calf skin acetone was more marked after exposure to UV-irradiation at 254 nm, subsequent experiments were carried out at this wavelength. The data in Fig. 4 show the effects of the two cyclic antibiotics on the transformation of ^{14}C -arachidonic acid into prostaglandins after exposure to UV-irradiation. Demeclocycline as with its effect on the oxygenation reaction enhanced the biosynthesis of total primary prostaglandins from arachidonic acid approximately 53%. Pre-irradiation of the antibiotics before incubation with arachidonic acid resulted in similar enhancement of prostaglandin biosynthesis.

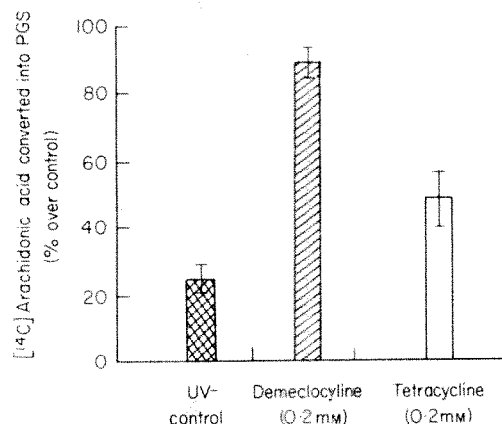


FIGURE 4. Effects of antibiotics and UV-irradiation on the transformation of arachidonic into prostaglandins by fetal calf skin acetone powder.

The incubation conditions containing test substances were as described in Legend for Fig. 2(b). The mixture was placed 10 cm under the UV-lamp at a wavelength of 254 nm and incubated at 37°C for 10 min. The data are from the mean of duplicate determinations from 3 experiments. The vertical bars represent ranges. Cross-hatched column, [¹⁴C]ARA + UV - 254 + ENZ; hatched column [¹⁴C]ARA + DMC + UV - 254 + ENZ; open column [¹⁴C]ARA + TC + UV - 254 + ENZ.

Effect of various antibiotics and UV-irradiation (254 nm) on the biosynthesis of prostaglandins from [¹⁴C]-arachidonic acid

In subsequent experiments, antibiotics without known photoreactive properties were tested *in vitro* with or without exposure to UV-irradiation at 254 nm on the oxygenation of arachidonic acid and the transformation of [^{1-¹⁴C}]-arachidonic acid into prostaglandins. The data in Table 1 show that after UV-irradiation at 254 nm, clindamycin, chloramphenicol and sulfacytine had no significant effect either on the oxygenation of arachidonic acid or on the biosynthesis of prostaglandins from arachidonic acid. These substances also had no effect in the absence of UV-irradiation. Chloram-

TABLE 1. The effects of various antibiotics and UV-irradiation (254 nm) on the biosynthesis of prostaglandins by fetal calf skin. Incubations and experimental procedure are as described in Fig. 2

Compounds	Oxygenation of arachidonic acid (% over control)*	Formation of prostaglandins from arachidonic acid (% over control)†
Photosensitizers:		
Demeclocycline	112.0	88
Tetracycline	58.0	47
Non-photosensitizers:		
Clindamycin	21	14
Chloramphenicol	8	Below control
Sulfacytine	2	0

* Assay based on oxygen consumption. Control is taken as 0.

† Assay based on total ¹⁴C-PGs formed from [^{1-¹⁴C}]-arachidonic acid.

phenicol exerted less than 10% inhibitory effect on the biosynthesis of prostaglandins, the significance of which is presently unclear.

DISCUSSION

The present data have demonstrated that exposure of cyclic antibiotics such as demeclocycline and tetracycline to UV-irradiation results in the *in vitro* enhancement of the oxygenation of arachidonic acid and the biosynthesis of prostaglandins by fetal calf skin. The effects are particularly more evident at 254 nm (UVC) than at 360 nm (UVA). Although our present data indicate only the determination of the primary prostaglandins, we do not exclude the formation of the cyclic endoperoxides or other products during shorter incubation periods. Our interest was to identify the more stable prostaglandins already known to be involved in cutaneous inflammatory reactions. These results are similar to a previous report from this laboratory (Lord *et al.*, 1976) on the effects of UV-irradiation on 8-methoxypsoralen, a photosensitizer currently in use for the management of psoriatic lesions. Although the precise mechanism for the enhancement of prostaglandin biosynthesis *in vitro* by UV-irradiation plus the photosensitizers is not fully clear, it is interesting to note that the exposure of unsaturated fatty acids to UV-irradiation is known to result in light-initiated cyclic peroxidation (Heath & Packer, 1968). Thus, it is likely that in our *in vitro* experiments, exposure of arachidonic acid (a polyunsaturated fatty acid) to UV-irradiation resulted in increased formation of an extremely reactive free-radical fatty acid with a strong affinity for oxygen and the cyclo-oxygenase. Likewise, the cyclic antibiotics on exposure to UV-irradiation very likely resulted in the formation of reactive photoproducts which would further enhance the cyclic peroxidation of the arachidonic acid. For instance, the formation of photoproducts after UV-irradiation of 8-methoxypsoralen (a photosensitizer) with a loss of characteristic peaks and valleys of spectrum has been described (Schonberg *et al.*, 1951, 1954; Lerner, Denton & Fitzpatrick, 1953). It is therefore reasonable to speculate that the cumulative effects of the UV-irradiation and the photo-products from the antibiotics may have caused the enhancement of the oxidative cyclization of arachidonic acid *in vitro* into peroxy acids and into prostaglandins. Experiments are being designed to delineate whether or not these occur. If this is the case, the significance of this study is to implicate certain chemical substances which on exposure to UV-irradiation could act as potential modulators of oxidative cyclization of released arachidonic acid and the eventual formation of prostaglandins.

Although our present data did not establish whether or not these reactions take place *in vivo*, it is nonetheless interesting to note that several studies have been reported that demethylchlortetracycline, a more potent and consistent photosensitizer than tetracycline (Blank, Cullen & Catalano, 1968; Frost, *et al.*, 1971), is associated with cutaneous inflammatory reaction. Furthermore, our data showed that non-photosensitizing antibiotics such as clindamycin, chloramphenol and sulfacytine had no effect *in vitro* on the oxidative cyclization of arachidonic acid and the biosynthesis of prostaglandins. It seems reasonable from these studies to suggest that the *in vivo* inflammatory reactions associated with the above cyclic antibiotics after exposure of subjects to UV irradiation may be due at least in part to their enhancement of the early cascade of biochemical reactions in the skin to form prostaglandins.

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REFERENCES

- BLANK, H., CULLEN, S.I. & CATALANO, P.M. (1968) Photosensitivity studies with demethylchlortetracycline and dioxycycline. *Archives of Dermatology*, **97**, 1.
- CULLEN, S.I., CATALANO, P.M. & HELFMAN, R.J. (1966) Tetracycline sun sensitivity. *Archives of Dermatology*, **93**, 77.
- FALK, M.S. (1960) Light sensitivity due to demethylchlortetracycline. *Journal of American Medical Association*, **172**, 1156.
- FROST, P., WEINSTEIN, G.D. & GOMEZ. (1971) Methacycline and demeclocycline in relation to sunlight. *Journal of American Medical Association*, **216**, 326.
- GREAVES, M.N. & SONDERGAARD, J. (1970) Pharmacologic agents released in ultraviolet inflammation studied by continuous skin perfusion. *Journal of Investigative Dermatology*, **54**, 365.
- GREAVES, M.W. & McDONALD, W. (1972) Prostaglandin biosynthesis by human skin and inhibition by corticosteroids. *British Journal of Pharmacology*, **46**, 172.
- JONSSON, C.D. & ANGAARD, E. (1972) Biosynthesis and metabolism of prostaglandin E₂ in human skin. *Scandinavian Journal of Clinical Investigation*, **29**, 289.
- LERNER, A.B., DENTON, C.R. & FITZPATRICK, T.B. (1953) Clinical and experimental studies with 8-methoxy psoralen in vitiligo. *Journal of Investigative Dermatology*, **20**, 299.
- LORD, J.T., ZIBOH, V.A., POITIER, J., LEGGET, G. & PENNEYS, N.S. (1976) The effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins. *British Journal of Dermatology*, **95**, 397.
- MATHUR, G.P. & GHANDI, V.M. (1972) Prostaglandin in human and albino rat skin. *Journal of Investigative Dermatology*, **58**, 291.
- PLUMMER, N.A., HENSBY, C.N., CAMP, R.D., WARIN, A.P. & GREAVES, M.W. (1977) Activation of arachidonate cascade in human skin inflamed by irradiation with UVC and the effects of indomethacin. *Journal of Investigative Dermatology*, **68**, 246.
- SCHONBERG, A., LATIF, N., MOUBASHER, R. & SINA, A. (1954) Photochemical reactions in sunlight. Part XVI. (A) Photoreduction of phenylglyoxylic acid. (B) Photoreactions between aldehydes and O-quinones. (C) Reactions between O-quinones and ethylenes in the dark and in the light. *Journal of the Chemical Society*, **1364**.
- SCHORR, W.F. & MONASH, S. (1963) Photo-irradiation studies on two tetracyclines. *Archives of Dermatology*, **87**, 134.
- SMITH, W.L. & LANDS, W.E. (1971) Stimulation and blockade of prostaglandin biosynthesis. *Journal of Biological Chemistry*, **246**, 6700.
- SNYDER, D.S. & EAGLSTEIN, W. (1974) Intradermal and prostaglandin agents and sunburn. *Journal of Investigative Dermatology*, **62**, 47.
- VAN DORP, D. (1971) Recent developments in the biosynthesis and the analysis of prostaglandins. *Annals of the New York Academy of Sciences*, **180**, 181.
- WALLACH, D.O. & DANIELS, E.G. (1971) Properties of a novel preparation of prostaglandin synthetase from sheep seminal vesicles. *Biochimica et Biophysica Acta*, **231**, 445.
- ZIBOH, V.A. (1973) Biosynthesis of prostaglandin E₂ in human skin: Subcellular localization and inhibition of unsaturated fatty acids and anti-inflammatory drugs. *Journal of Lipid Research*, **14**, 337.
- ZIBOH, V.A. & HSIA, S.L. (1971) Prostaglandin E₂: Biosynthesis and effects of glucose metabolism in rat skin. *Archives of Biochemistry and Biophysics*, **146**, 100.

Personnel radiation dosimetry in drug photosensitivity: field study of patients on phenothiazine therapy

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SUMMARY

A lapel badge dosimeter sensitive to short wave UVR has been used in a preliminary trial to survey photosensitivity in psychiatric patients on phenothiazine therapy. The badge was found easy to use and the results suggest that chlorpromazine is more photosensitizing than other phenothiazines.

About 60 oral drugs which appear to cause skin photosensitivity are listed in the returns to the Committee on Safety of Medicines in the UK. The diagnosis of photosensitivity is usually made clinically from the history of administration of a drug followed by the development, after exposure to sunlight, of symptoms and lesions on the exposed skin which resolve if either the drug or solar exposure is stopped. We believe, however, that a reasonably certain diagnosis of photosensitivity is difficult to make without more objective data. The dose of a therapeutic drug is normally known where a patient suffers an adverse effect, but where this effect is photosensitivity, the amount of sunlight received and its spectral composition are unknown. These factors are presumably just as important as the drug and its dose in assessing diagnosis and in planning treatment and prevention.

With the development by Davis, Deane & Diffey (1976) of a simple ultraviolet radiation (UVR) dosimeter which individual patients can use as a badge, a new approach to studying photosensitizing drugs is possible. The dosimeter is in the form of a film of plastic, polysulphone. It has already been put to field trial with promising results by Challoner *et al.* (1976) in normal persons exposed to different amounts of sunlight. We here record a study with the dosimeters on patients taking phenothiazines, which are known photosensitizers (Labhardt, 1954; Schulz, Wiskemann & Wulf, 1956). Our aim was to assess the value of the dosimeter under field conditions within a closed community in which a high prevalence of photosensitivity symptoms would be expected, to correlate these symptoms with solar exposure and to compare various methods of assessing solar exposure.

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The action spectrum for skin lesions from chlorpromazine appears to lie in a spectral region of longer wavelength than that causing normal sunburn (Schulz *et al.*, 1956; Ison & Blank, 1967). In humans this may extend from 300 to 400 nm and even beyond into the visible spectrum (Kligman & Breit, 1968). According to monochromator irradiation studies in mice photosensitized with chlorpromazine, spectral reactivity is within 300–400 nm, maximally at about 310–330 nm (Hunter, Bhutani & Magnus, 1970). The wavelengths that activate the polysulphone film badge are 330 nm and below, i.e. there is some overlap of the wavelength response of the film with the probable action spectrum for chlorpromazine photosensitivity. The maximum solar UVR in the spectral range 310–330 nm that the film can record is about 2 J.cm^{-2} (Diffey, 1977). This dose would be easily exceeded in continuous solar exposure during 1 week in the summer and might be exceeded in intermittent exposures. We decided, nevertheless, for simplicity, to have the patients in our trial wear only one badge per week and to accept the risk of some badge overexposure with the loss of data on UVR exposure.

Although the photosensitizing effects of chlorpromazine are the best known, other phenothiazines thought to have a similar adverse effect are commonly used in psychiatry. Therefore we decided to include these in the study although little is known about their photosensitizing properties. For the present experiments we have assumed that their photosensitizing action spectra are similar to that of chlorpromazine.

MATERIALS AND METHODS

The polysulphone film badge dosimeter was used; the UVR dose is found by measuring the change in optical absorbance of the film at 330 nm which results from UVR exposure (Davis *et al.*, 1976).

A diary card (Fig. 1) was kept for every patient for each of the 8 weeks of the study period, 4 July–28 August, 1976. Five different psychiatric hospitals, each one near a meteorological station where

TRANQUILLISER PHOTOSENSITIVITY STUDY									
NAME:									
Age:		Sex:		RECORD No.:					
Week Commencing:		BADGE No.:							
Day	Itching	Burning	Pain	Redness	Swelling	Hours out of doors	Hours out of doors in bright sunshine	Drug and dose	Remarks
Sun.	Y N	Y N	Y N	Y N	Y N				
Mon.	Y N	Y N	Y N	Y N	Y N				
Tues.	Y N	Y N	Y N	Y N	Y N				
Wed.	Y N	Y N	Y N	Y N	Y N				
Thur.	Y N	Y N	Y N	Y N	Y N				
Fri.	Y N	Y N	Y N	Y N	Y N				
Sat.	Y N	Y N	Y N	Y N	Y N				

Please put a circle around Y for Yes or N for No on each day to indicate whether symptoms have occurred or not.

Please return this sheet and light monitor film in plastic mount as soon as possible at end of week in envelope provided.

FIGURE 1. The diary card.

daily sunshine hours were recorded, were chosen. They lay within latitude 51–53°N; four were in Southern England, and one in Eire.

Hospital staff were asked to select 5 patients on phenothiazine therapy and provide the data requested on the diary card (Fig. 1). Any patient who was discharged was to be replaced by another suitable patient who joined in the trial at that point.

The dosimeter badges were worn all day by the patients; one badge per patient per week. At the end of each week, the badges were posted to one of us (A.D.) for measurement of the dose of UVR received.

RESULTS

Patients

Eighteen men and 14 women, who were under treatment for schizophrenic conditions, took part. The average age was 48 years, range 28–65 years.

Symptoms

Of a possible 1400 patient-days, 1355 were suitable for analysis and symptoms occurred in 300 instances, viz. 22%. The total prevalence of the various symptoms was: erythema, 62%; burning, 15%; itching, 14%; pain, 6%; oedema, 3%.

TABLE 1. Phenothiazine drugs prescribed and their dose

Drug	Dose range, (mg)	No. of patient-weeks studied
Chlorpromazine	500–800/day	92
Fluphenazine decanoate	25 every 3 weeks–50 once weekly	42
Thioridazine	100–450/day	42
Trifluoperazine	10–100/day	26
Perphenazine	8/day	8
Promazine	300/day	8
Fluphenazine HCl	75–100 every 2 weeks	6

Drugs

The drugs with dose prescribed are listed in order of frequency used (Table 1). Chlorpromazine was the most commonly used. Some patients received several of the 7 drugs listed, together or in combination with others not recorded as photosensitizing, e.g. flupenthixol.

Correlation between methods of estimating solar exposure

Product-moment correlation coefficients were calculated between:

- (1) Staff records for hours patients spent outdoors per week.
- (2) Staff records for hours patients spent in direct sunlight per week.
- (3) Badge dose per week (where film was not overexposed).
- (4) Local Met. Office hours of sunshine per week expressed as daily average.

The coefficients are shown in Table 2. The Met. Office sunshine hours neither correlated with hours outdoors (as recorded by the hospital staff) nor with badge dose. But hours outdoors correlated significantly with badge dose and with hours in direct sunlight ($P < 0.001$ in each case). Furthermore, hours in direct sunlight correlated with badge dose and with Met. Office sunshine hours ($P < 0.01$ and 0.001 respectively).

TABLE 2. Comparison of data for methods of assessing solar exposure in patients: Product-moment correlation coefficients

	No. of pairs	Correlation coefficient	Significance
(a) Hours outdoors (1) and badge dose (not overexposed) (2)	84	$r_{12}=0.48$	$p<0.001$
(b) Hours outdoors (1) and met. hours sunshine (3)	141	$r_{13}=0.15$	N.S.
(c) Badge dose (not overexposed) (2) and met. hours sunshine (3)	120	$r_{23}=0.17$	N.S.
(d) Hours outdoors (1) and hours out in direct sunlight (4)	124	$r_{14}=0.54$	$p<0.001$
(e) Badge dose (not overexposed) (2) and hours out in direct sunlight (4)	78	$r_{24}=0.32$	$p<0.01$
(f) Met. hours sunshine (3) and hours out in direct sunlight (4)	142	$r_{34}=0.30$	$p<0.001$

TABLE 3. Patient and solar exposure; data from the five different psychiatric hospitals

	A	B	Hospitals C	D	E
No. of different patients studied	7	5	8	5	7
No. of patient-weeks	40	40	39	39	39
No. of weeks showing complete record of 'hours out of doors'	32	40	23	14	37
No. of badge observations available	40	39	39	38	36
No. of overexposed badges	24 (60%)	10 (26%)	15 (38%)	0	19 (53%)

Over-exposed dosimeter badge films

The incidence of over-exposed films, i.e. with a dose $\geq 2 \text{ J.cm}^{-2} \text{ week}^{-1}$, varied with institution; it was nil at Hospital D, but 60% at Hospital A (see Table 3). Met. Office sunshine hours corresponding to overexposed films were compared with those corresponding to film doses less than $2 \text{ J.cm}^{-2} \text{ week}^{-1}$. The difference was not significant, although the Met. Office hours associated with underexposed films were slightly higher than those with overexposed films (see Table 4).

Table 4 shows that the patients whose dosimeter badges were overexposed spent significantly longer outdoors ($P<0.001$) while the time they spent out in direct sunlight was also significantly longer ($P<0.02$) than that recorded for patients whose badge doses showed $<2 \text{ J.cm}^{-2} \text{ week}^{-1}$.

Correlation of symptoms with solar exposure

A binary symptom score was formed by taking 0 to denote no symptoms recorded during the week and

TABLE 4. Dosimeter badges, overexposed and not overexposed; relationship to other methods of assessing patients' solar exposure

		Badge doses $\geq 2 \text{ J.cm}^{-2} \text{ wk}^{-1}$	Badge doses $< 2 \text{ J.cm}^{-2} \text{ wk}^{-1}$	Significance	
				<i>T</i>	<i>P</i>
(a) Time spent out- doors per week	No. of patient-weeks	57	85	5.13	< 0.001
	Mean time (h)	30.5	21.1		
	s.d. (h)	10.0	11.1		
(b) Time spent in direct sunlight per week	No. of patient-weeks	58	78	2.6	< 0.02
	Mean time (h)	15.4	11.7		
	s.d. (h)	8.4	8.3		
(c) Local Met. Office sunshine hours per day	No. of patient-weeks	67	125	1.22	N.S.
	Mean time (h)	8.0	8.5		
	s.d. (h)	3.0	2.6		

TABLE 5. Correlation of methods of assessing solar exposure in patients; point biserial correlation coefficients

		Patient- weeks	Mean	r_{pb}	Significance
(a) Symptom score and badge dose ($\text{J.cm}^{-2} \text{ week}^{-1}$) where badges were not overexposed	Score = 0	75	0.7	0.33	$P < 0.001$
	Score = 1	49	1.1		
(b) Symptom score and met hours sunshine (h.day ⁻¹)	Score = 0	111	7.8	0.26	$P < 0.001$
	Score = 1	85	9.2		
(c) Symptom score and hours out in direct sunlight (h.week ⁻¹)	Score = 0	81	11.2	0.22	$P < 0.01$
	Score = 1	68	15.0		
(d) Symptom score and hours outdoors (h.week ⁻¹)	Score = 0	81	23.6	0.14	N.S.
	Score = 1	65	26.8		

1 to denote one or more symptoms during the week. A point biserial correlation (Kendall & Stuart, 1973) was made between the symptom scores and the badges in which film dose was below $2 \text{ J.cm}^{-2} \text{ week}^{-1}$ (Table 5a). These were significantly correlated ($P < 0.001$). Similarly, weekly symptom scores were significantly correlated with weekly Met. Office sunshine hours ($P < 0.001$) and with hours in direct sunlight ($P < 0.01$) (Tables 5b and c). Symptom score was not correlated with hours per week spent outdoors (Table 5d). In all cases of significant correlation, the presence of symptoms was associated with higher weekly means of weekly badge dose, of Met. Office sunshine hours per week and hours in direct sunlight respectively than where there were no symptoms.

Correlation between symptoms and drugs used

The data were suitable only for comparing patients who had had chlorpromazine as part of their treatment with those who had had other phenothiazines. Their symptom records constitute two independent samples in each of which there are multiple observations from each patient. Accordingly, a photosensitivity score was defined by allotting to each patient the number of weeks out of 8 in which symptoms had been recorded, and the data were thus transformed to two independent sets of scores,

TABLE 6. Comparison of scores and badge doses of patients on chlorpromazine with those on other phenothiazines

(a) Photosensitivity scores	Chlorpromazine	$n_1 = 13$, Median score 4.0,	$U = 36$, $P < 0.05$
	Other phenothiazines	$n_2 = 12$, Median score 2.5,	
(b) Overexposed badges	Chlorpromazine	3 out of 13 patients showed 3 or more overexposed badges	$\chi^2 = 3.3$, N.S.
	Other phenothiazines	7 out of 12 patients showed 3 or more overexposed badges	

TABLE 7. Comparison of exposure data of patients on chlorpromazine with those on other phenothiazines

		Patient-weeks	Mean	s.d.	<i>t</i>	Significance
(a) Badge dose ($J.cm^{-2}.wk^{-1}$) where badge not overexposed	Chlorpromazine	69	0.9	0.5	1.5	N.S.
	Other phenothiazines	55	0.8	0.5		
(b) Hours outdoors ($h.wk^{-1}$)	Chlorpromazine	79	26.7	9.3	1.9	N.S.
	Other phenothiazines	66	23.1	13.6		
(c) Hours in direct sunlight ($h.wk^{-1}$)	Chlorpromazine	80	13.8	8.6	1.3	N.S.
	Other phenothiazines	66	11.9	8.5		
(d) Met. office sunshine hours ($h.day^{-1}$)	Chlorpromazine	104	8.8	2.5	3.0	$P < 0.01$
	Other phenothiazines	96	7.5	3.6		

which were compared using the Mann-Whitney U test (see Table 6a). The chlorpromazine-treated patients showed higher photosensitivity scores than the other patients and the difference between the two treatments groups was significant ($P < 0.05$). Since this might have been explained by differences in solar exposure the data on exposure were examined in detail (Tables 6b and 7a-b).

The chlorpromazine-treated patients did not show a significant difference from the patients treated with other drugs in the numbers of overexposed badges, time spent outdoors per week, badge doses of UVR or time spent out in direct sunlight (Tables 6b and 7a-c). Those who had chlorpromazine, however, were in places with significantly more available sunlight, as measured by Met. Office hours of sunshine (Table 7d), and they could have had higher solar exposures than the non-chlorpromazine group had they been outdoors at all times.

DISCUSSION

Our main aim was to test whether a patient's solar exposure can be measured with a lapel dosimeter. We used psychiatric patients because their light-induced symptoms offered a yardstick against which to measure the dosimeter's performance. Our findings accord with the clinical belief that phenothiazines are probably photosensitizing and that chlorpromazine is more active than other drugs in this group.

The attitude of the hospital staff to phenothiazine, and in particular to chlorpromazine, photosensitivity may have influenced our results, although we asked that patients were not to modify their usual habits in solar exposure. Practice varied from one hospital to another; in one it was usual to discourage undue exposure of fair-skinned patients. As the staff knew of the nature of our project, some sunlight protection might have been attempted in three other hospitals. In the fifth hospital, no special measures were taken to avoid photosensitivity even though they took part in the project.

The experience gained in this trial will help in future projects. For instance, some diary card data had to be discarded because of ambiguous entries; our instructions had probably been insufficiently clear. Also the methods used to collect certain data have resulted in some uncertainties. First, the symptoms of photosensitivity should ideally have been assessed by a dermatologist at the hospital, but this was not possible. Secondly, the dosimeter action spectrum only partially matched that of the biological response under study; it is probably too narrow for chlorpromazine skin photosensitivity, a little too wide for normal sunburn.

The higher photosensitivity score for chlorpromazine compared with other drugs was not due to chlorpromazine patients being exposed to more sunlight than the non-chlorpromazine treated, according to the badge dose and records for hours outdoors and in direct sunlight (Table 7). The significant difference between the two treatment groups found in Met. Office sunshine hours may be due to the fact that chlorpromazine was hardly used at Hospital A which also had the cloudiest weather.

The badge vs other methods of assessing solar exposure

The weekly score for symptoms and signs correlated significantly with the weekly badge dose for UVR (Table 5a). Symptom scores also correlated with Met. Office sunshine hours (Table 5b) and with hours spent in direct sunlight (Table 5c). Thus these three methods of assessing symptoms against solar exposure in patients appear to agree well, as would be expected. But symptom score and hours outdoors did not correlate (Table 5d); this is plausible, for the time outdoors in our climate will include many periods of overcast weather.

Next, the hours outdoors and the hours spent by the patients in direct sunlight (as recorded by the staff) correlated significantly with the badge dose (Table 2a and d). Even when badges were overexposed, the mean hours outdoors were significantly higher than the mean when the badges were not overexposed (Table 4).

The lack of correlation between Met. Office sunshine hours and badge dose (Table 2c) and between Met. Office hours and hours recorded outdoors (Table 2b) is not surprising. Patients will not be able to go outdoors just because the sun is shining if this interferes with hospital routine. In view of this, the finding, mentioned above, that chlorpromazine-treated patients were in locations with significantly higher Met. Office sunshine hours, as against the patients not on this drug, can probably be ignored as irrelevant.

Again the lack of correlation between badge dose and Met. Office sunshine hours is to be expected when one considers how the latter are obtained. The Campbell-Stokes sunshine hours recorder is used. It is activated chiefly by the visible and infra-red portion of the solar spectrum, hardly at all by the UVR. As it cannot distinguish between solar radiation with UVR or without, its response to an hour of early morning or late afternoon sunshine and to one around midday may be very similar, although midday sunshine has more UVR. The polysulphone film badge on the other hand is sensitive to the region of the solar spectrum at and below 330 nm and its response is proportional to dose. Also, at least half of the radiation in this wavelength range comes not directly from the sun but is scattered from the dome of the sky (Davis *et al.*, 1977). Hence the polysulphone badge can still show a significant response in conditions when the Met. Office records no sunshine, for the Campbell-Stokes instrument records direct sunlight only and when a cloud passes in front of it, it does not register. Of the 5

techniques for assessing UVR sunlight exposure, the dosimeter badge is to be preferred as the most meaningful and practical. Furthermore, it is the method which gives a quantitative measure of what an individual was exposed to.

Polysulphone film

The polysulphone film proved simple to use for staff and acceptable to patients. Very few badge films had to be discarded owing to damage. The films were returned by post to one of us (A.D.) for processing, a procedure convenient for field trials.

We conclude that a polysulphone film badge is useful as a personnel dosimeter in field trials, epidemiological and similar studies, where information on UVR exposure to the skin is required. Such studies, apart from the more obvious dermatological and industrial applications, would include observations in regions where skin cancer epidemiology and surveys on Vitamin D status are of importance.

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REFERENCES

- CHALLONER, A.V.J., CORLESS, D., DAVIS, A., DEANE, G.H.W., DIFFEY, B.L., GUPTA, S.P. & MAGNUS, I.A. (1976) Personnel monitoring of exposure to ultraviolet radiation. *Clinical and Experimental Dermatology*, **1**, 175.
- DAVIS, A., DEANE, G.H.W. & DIFFEY, B.L. (1976) Possible dosimeter for ultraviolet radiation. *Nature*, **261**, 169.
- DAVIS, A., HOWES, B.V. & LEDBURY, K.J. (1977) Solar ultraviolet radiation measurements made with polysulphone at a site in the United Kingdom. *P.E.R.M.E. Technical Report No. 52*.
- DIFFEY, B.L. (1977) Personal communication.
- HUNTER, J.A.A., BHUTANI, L.K. & MAGNUS, I.A. (1970) Chlorpromazine photosensitivity in mice: its action spectrum and the effect of anti-inflammatory agents. *British Journal of Dermatology*, **82**, 157.
- ISON, A. & BLANK, H. (1967) Testing drug phototoxicity in mice. *Journal of Investigative Dermatology*, **49**, 508.
- KENDALL, M.G. & STUART, A. (1973) Point biserial correlation. In: *The advanced theory of statistics*, 3rd edn., Vol. 2, p. 323. Charles Griffin & Co. Ltd., London.
- KLIGMAN, A.M. & BREIT, R. (1968) The identification of phototoxic drugs by human assay. *Journal of Investigative Dermatology*, **51**, 90.
- LABHARDT, F. (1954) Technik, Nebenerscheinungen und Komplikationen der Largactiltherapie. *Schweizer Archiv für Neurologie, Neurochirurgie und Psychiatrie*, **73**, 338.
- SCHULZ, K.H., WISKEMANN, A. & WULF, K. (1956) Klinische und experimentelle Untersuchungen über die photodynamische Wirksamkeit von Phenothiazenderivaten, insbesondere von Megaphen. *Archiv für Klinische und Experimentelle Dermatologie*, **202**, 285.

ADDENDUM

Ljunggren & Möller (*Acta dermato-venereologica*, 1977, **57**, 325) have recently shown in the mouse that chlorpromazine is the most active photosensitizer of the phenothiazines used in clinical practice. This corroborates our finding.

Tests to establish the diagnosis in cholinergic urticaria

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SUMMARY

Methods for establishing the diagnosis in cholinergic urticaria are reviewed and compared. Provocation by exercise or hot bath is more effective than local provocation by intradermal tests. Of the intradermal tests, responses to nicotine acid tartrate and acetyl β methylcholine chloride are positive only in patients with the more severe, readily induced eruptions. Furthermore, intradermal testing is not always reproducible in the same patient. In our patients, exercise proved the simplest, most effective diagnostic test.

Cholinergic urticaria is common. For example, Herxheimer found an incidence of 20% with symptoms suggestive of cholinergic urticaria in a survey of 1083 university students (Calnan, 1964). The diagnosis is suggested by a history of a short-lived, pruritic rash evoked by exercise, heating or psychic stimuli, and is established by observing the typical eruption. Unlike other forms of urticaria the weal is smaller, rarely exceeding 5 mm in diameter and arises on a bright red flare. Diagnostic difficulties may arise because of its transience and because other types of urticaria including chronic urticaria and dermatographism often seem to be worsened under similar circumstances. The characteristic rash of cholinergic urticaria is reproducible and this property can therefore be used to confirm the diagnosis. Although the nature of the relationship remains speculative, the rash is usually associated with the provocation of eccrine sweating. Accordingly, diagnostic tests for cholinergic urticaria involve stimulation of sweating by local or central means. The aim of this study is therefore to review the available diagnostic techniques and to clarify their role, if any, in the confirmation of the diagnosis of cholinergic urticaria as an essential first step in pharmacological characterization of this disorder.

PATIENTS AND METHODS

Patients

Twenty patients (15–38 years old; 11 males, 9 females) were referred for the investigation. On

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clinical grounds the diagnosis of cholinergic urticaria was excluded in 8 of these. This group proved to have dermatographism (4), chronic urticaria (2) or pruritus (2) but were retained in the study as a control group. Six healthy young adults (aged 17–29 years; 3 male, 3 female) were also tested. In the 12 patients with cholinergic urticaria the rash varied from mild to disabling. All patients had been free of the eruption and antihistamine treatment had been withdrawn during the 24 h prior to testing. Patients were investigated as outpatients or inpatients. The ambient temperature was kept constant at about 22°C. Sweating was confirmed in all patients by applying starch paper painted with 3% iodine in ethanol.

Methods

The following provocative tests were used in all patients and all tests, including intradermal tests, were repeated on another occasion.

Hot bath. Sweating occurs if the central temperature rises 1°C (Johnson & Spalding, 1974). Immersion of the hand in water at 43°C will achieve sweating but is capricious (Morgan, 1953). In this study the oral temperature was raised 1–1.5°C by half immersion in a bath at 43°C. The patient was examined out of the bath for 30 min after profuse sweating had been induced.

Exercise. The patients ran on the spot in warm clothes until sweating was induced.

Local pharmacological provocative tests

The back proved to yield the most reproducible results and was therefore used for all intradermal tests. Responses to cholinergic and nicotinic stimulation and to hypertonic saline solutions were evaluated. Each injection was of 0.1 ml volume using 0.154 mol/l saline as a diluent. Larger volumes were more painful whilst smaller volumes often gave false negative results. Care was taken to avoid injection of a single site more than once. All patients received control injections of the diluent alone. The development of satellite weals around the injection site was considered a positive response.

(i) *Acetyl B methylcholine chloride* (mecholy). Acetylcholine is unstable in solution (Goodman & Gilman, 1975) and is rapidly hydrolysed by cholinesterase. Mecholy is more suitable for routine testing because it is stable and has a more prolonged action. Injection of mecholy produces a weal and a flare. The development of satellite weals is considered to establish a diagnosis of cholinergic urticaria (Kaplan, 1976). The injection itself raises an initial bleb. In cholinergic urticaria the size of this bleb often increases but this is difficult to judge and is not a specific sign as it can occur in patients with dermatographism. Mecholy was injected in concentrations of 5.1×10^{-4} and 5.1×10^{-5} mol/l.

(ii) *Nicotine acid tartrate* (nicotine). Rothman & Coon (1940) showed that an intradermal injection of nicotine produces a flare, sweating and a pilomotor reflex in normal subjects. However, in cholinergic urticaria Herxheimer (1956) and Illig (1967) demonstrated satellite weals in response to local injection of this agent. Its value as a diagnostic test in cholinergic urticaria was therefore assessed. Nicotine acid tartrate was injected in a concentration of 2.0×10^{-7} mol/l. Concentrations above and below this were found to be ineffective.

Hypertonic saline. Wada (1951) showed that axon reflex sweating and erythema could be induced by hypertonic saline, a 0.684 mol/l solution being the optimum concentration. Patients with cholinergic urticaria developed satellite weals (Herxheimer, 1956) and so this agent was also tested.

TABLE 1. Details of 12 patients with cholinergic urticaria

Patient	Age and sex	Severity and duration	Usual provocative stimuli				Results of diagnostic test			
			Hot bath	Exercise	Stress	Other	Hot bath	Exercise	Mecholyl	NAT
1	F/23	1†	+	+		Hot food	+	+	-	-
2	M/20	4‡	+				+	+	-	-
3	F/28	16§	+	+	+		+	+	±	+
4	M/33	3†			+		-	-	-	-
5	M/35	2†	+	+		Hot food	+	+	+	-
6	F/24	8§	+	+	+	Hot food	+	+	+	+
7	M/18	2†	+	+	+	Hot food	+	+	-	-
8	F/29	4§	+	+	+		+	+	±	+
9	F/15	5‡	+	+	+		+	+	-	-
10	M/16	1‡	+	+			+	+	±	-
11	M/18*	2‡	+	+			+	+	±	+
12	F/25	12‡	+			Water contact	+	+	+	-

* Also has cold urticaria.

† Mild; ‡ Moderate; § Severe.

+ Consistent positive; - Consistent negative; ± Repeat tests had differing results. All control 0.154 mol/l saline injections normal. NAT = nicotine acid tartrate.

RESULTS

None of the 14 subjects in the two control groups showed positive responses to any of the tests. The results in the 12 patients in whom the diagnosis of cholinergic urticaria was made on clinical grounds are shown in Table 1. One patient (patient 8) had systemic symptoms during attacks. Another patient (patient 11) also had cold urticaria. Only one patient failed to respond to any of the battery of tests. This patient's eruption was mild and provoked only by emotional stress. All the remaining patients gave a positive response to exercise, developing typical wealing within 20 min of onset of sweating. Immersion in a hot bath evoked a positive response in 10 patients usually within 20 min of immersion. Non-immersed skin had more lesions than immersed skin.

Intradermal testing was positive only in those patients with severe cholinergic urticaria and was often not reproducible (see Table 1). Six patients gave positive reactions to mecholyl; of these only three (patients 6, 11 and 12) had consistently positive responses on repeated testing. In those patients with positive reactions mecholyl was equally effective at the 5.1×10^{-4} and 5.1×10^{-5} mol/l concentration, with satellite weals developing within 10 min of challenge. Positive responses to nicotine acid tartrate 2.0×10^{-7} mol/l were also confined to the severest cases with four patients giving positive local reactions (patients 3, 6, 8 and 11). Hypertonic saline gave positive reactions in only two severe cases. Mecholyl and hypertonic saline were painful in some people.

DISCUSSION

We agree with other authors (Warin & Champion, 1974) that provocative testing by exercise or a hot bath confirms the diagnosis in most patients with cholinergic urticaria. Only one patient with a history strongly suggestive of cholinergic urticaria failed to respond. This patient is known to have cholinergic urticaria because the typical rash was observed to occur in association with an emotional response to the prospect of multiple skin tests. The patient was able to exercise and take sauna baths without discomfort although anxiety frequently caused attacks. Both this patient, and a patient described by Kay & Maibach (1969) who had acquired anhidrosis associated with cholinergic urticaria, suggest that cholinergic urticaria does not depend on eccrine sweating, and raises the possibility that the wealing may be a response to increased sympathetic tone and its vascular consequences.

An intradermal diagnostic test for cholinergic urticaria, if sensitive and reliable, would obviate the inconvenience of vigorous exercise or immersion in a hot bath. A variety of tests have been advocated for this purpose. Randall (1947) induced direct sweating with local heat but this required special apparatus. We tried a physiotherapist's infra-red lamp but this was unsuccessful. Faradic axon stimulation will also induce localized sweating (Bickford, 1938) but this is painful and requires equipment that may not be readily available. Intradermal testing with small doses of nicotine, mecholyl or hypertonic saline is safe and simple, with minimum discomfort to the patients. However, in our patients these local tests were only positive in the more severe cases and in some individuals were not consistent when repeated on another occasion. Thus, of the tests examined, exercise and hot bathing remain the methods of choice in the diagnosis of cholinergic urticaria and have the advantage of reproducing the condition as it is experienced by the patient.

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REFERENCES

- BICKFORD, R.E. (1938) The mechanism of local sweating in response to faradism. *Clinical Science*, **3**, 337.
- CALNAN, C.D. (1964) Urticarial reactions. *British Medical Journal*, **ii**, 649.
- GOODMAN, L.S. & GILMAN, A. (editors) (1975) *The Pharmacological Basis of Therapeutics*, 5th edn, p. 467. MacMillan Publishing Co., New York.
- HERXHEIMER, A. (1956) The nervous pathway mediating cholinergic urticaria. *Clinical Science*, **15**, 195.
- JOHNSON, R.H. & SPALDING, J.M.K. (1974) *Disorders of the Autonomic Nervous System*, p. 181. Blackwell Scientific Publication, Oxford.
- ILLIG, L. & HEINICKE, A. (1967) Zur Pathogenese der cholinergischen Urticaria II. *Archiv für klinische und experimentelle Dermatologie*, **229**, 285.
- KAPLAN, A.P. & BEAVEN, M.A. (1976) *In vivo* studies of the pathogenesis of cold urticaria, cholinergic urticaria and vibration induced swelling. *Journal of Investigative Dermatology*, **67**, 327.
- KAY, D.M. & MAIBACH, H.I. (1969) Pruritus and acquired anhidrosis. Two unusual cases. *Archives of Dermatology*, **100**, 291.
- MORGAN, J.K. (1953) Observations on cholinergic urticaria. *Journal of Investigative Dermatology*, **21**, 173.
- RANDALL, W.C. (1947) Local sweat gland activity due to direct effects of radiant heat. *American Journal of Physiology*, **150**, 365.
- ROTHMAN, S. & COON, J.M. (1940) Axon reflex responses to acetylcholine in the skin. *Journal of Investigative Dermatology*, **3**, 79.
- WADA, M., ARAI, T., TAKAGAKI, T. & NAKAGAWA, T. (1951) Axon reflex mechanism in sweat responses to nicotine, acetylcholine and sodium chloride. *Journal of Applied Physiology*, **4**, 745.
- WARIN, R.P. & CHAMPION, R.H. (1974) Urticaria. In: *Major Problems in Dermatology* (Ed. by A.J. Rook), vol. 1, p. 140. W.B. Saunders Co., London.

Streptococcal impetigo and acute glomerulonephritis in children in Cairo*

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SUMMARY

Impetigo contagiosa in Cairo affected young children of both sexes, the face being the main site. Post-impetigo nephritis, confirmed by a low serum C3 level and by urinalysis, occurred in only 11% of cases. *Streptococcus pyogenes* strains were recovered from 84% of the skin lesions. Sixteen types were identified according to their T-protein, and most infections were associated with T3/B3264, T13/B3264, T5, T11, T12, T8/25/Imp 19 and T14/49; the majority of these types were also recovered from houseflies. The types isolated from cases of post-impetigo nephritis were T4 (M 60), T14/49 (M 49), T8/25/Imp 19 (mostly M 55) and T11. Seventy percent of the patients infected with T4 (M 60) and 40% of those infected with T14/49 (M 49) developed nephritis. Strains isolated from the skin bore a closer resemblance to those isolated from the nose than to those found in the throat. The ASO response was poor in uncomplicated impetigo but the titre rose more often in post-impetigo nephritis.

Impetigo contagiosa is an acute superficial infection of the skin caused mainly by *Streptococcus pyogenes* and *Staphylococcus aureus*. Wannamaker (1970) reported a marked difference between streptococcal skin infection and streptococcal throat infection from the clinical, bacteriological, epidemiological and serological points of view, but pointed out that both might be complicated by acute glomerulonephritis.

In certain areas, especially in tropical and subtropical climates, the skin is the most usual site of infection preceding nephritis. Epidemics of glomerulonephritis in which skin infections predominated occurred in Trinidad (Parker *et al.*, 1968) and in the Red Lake Indian Reservation in Minnesota (Anthony *et al.*, 1969; Ferrieri *et al.*, 1972; Kleinman, 1954).

For 2 years (May 1974–May 1976) we have studied the bacteriology of impetigo in Cairo and the prevalence there of post-impetigo glomerulonephritis.

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MATERIALS AND METHODS

Bacteriological methods

In this study, 627 children of both sexes, all less than 12 years of age, were seen at Cairo Skin and Venereal Diseases Hospital (Hod El Marsoud) with inflammation of the skin. They were subjected to a thorough clinical examination for clinical evidence of impetigo contagiosa in the form of bullae, pustules or crusts. These lesions were swabbed under as nearly aseptic conditions as possible. They were cleansed with ethanol and opened with a sterile needle, and then the base of the lesion was touched and smeared with sterile cotton-tipped applicators moistened with sterile saline. The nose and throat of every patient were also swabbed. Two blood agar plates were used for each specimen, one being incubated aerobically and the other anaerobically, and both were examined after 18 h and re-examined on the second day. Any colony suspected of being *Streptococcus pyogenes* was picked off and transferred to 3% blood broth and incubated overnight at 37°C. Two bottles of Todd-Hewitt broth were inoculated with samples from this blood broth. One, containing 50 ml, was incubated overnight at 37°C for grouping and M-typing, and the other, containing 10 ml, was incubated at 30°C for T-typing. A loopful of blood broth culture was inoculated onto blood agar plates and a two-unit bacitracin disc was applied for preliminary identification of Group A streptococci.

Beta-haemolytic streptococci were grouped by Lancefield's method of hot acid extraction and precipitation (Lancefield, 1933). *S. pyogenes* strains were M-typed by agglutinating the trypsinized cells with antisera of the following types (Griffith 1934): 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19, 22, 23, 25, 27, 28, 44, 47 and 49 and the provisional types Imp 19 and B3264*. M-typing of *S. pyogenes* strains isolated from cases of post-impetigo nephritis was carried out by the precipitation method of Swift *et al.* (1943) using sera of the following types: 1, 2, 3, 4, 5, 6, 8, 11, 12, 13, 14, 15, 17, 18, 19, 22, 23, 24, 25, 26, 29, 30, 31, 32, 33, 36, 37, 38, 39, 40, 41, 42, 43, 46, 47, 49, 51, 53, 55†, 56, 57, 58‡ and 60§.

Isolation of S. pyogenes from domestic flies (Musca domestica vicin a)

The housefly was transferred to a tube containing 3 ml Pike's medium (Pike, 1944), incubated at 37°C for 8 h and then subcultured on two blood agar plates. Beta-haemolytic streptococci were grouped and typed as previously described.

Urinalysis

The patient's urine was collected once weekly for three successive weeks. It was tested for the presence of protein by the heat coagulation test with 2% acetic acid. It was also examined microscopically for the presence of red blood cells (RBCs) and cellular and/or granular casts.

Immunological and serological tests

(1) *Estimation of serum C3 level.* Blood samples were taken from 50 apparently healthy children as controls and from all patients with impetigo contagiosa. Immunodiffusion C3 plates and protein serum B solution—for plotting the C3 reference curve—were purchased from Hoechst agency. The test was carried out as described in Hoechst publications.

(2) *Antistreptolysin O titre (ASO)* was estimated by the modified method of Rantz & Randall (1945).

* Typing sera were purchased from WHO Streptococcal Reference Laboratories, Czechoslovakia.

† & ‡ Sera were kindly provided by Dr R Facklam, CDC, Georgia.

§ Sera by Dr W. R. Maxted, CPHL, London.

RESULTS

Streptococcal impetigo in relation to age and sex

The majority of affected children (97%) were less than 10 years of age; 62% were less than 5 years, and 35% were between 5 and 10 years old. Girls outnumbered boys (320 and 307). Most lesions were located on the face (Table 1).

TABLE 1. Site of impetigo lesions

No. of Patients	Site of infection											
	Face only		Face and upper limbs		Face and lower limbs		Upper limbs		Lower limbs		All body	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
627	503	80.2	43	6.8	40	6.4	11	1.8	20	3.2	10	1.6

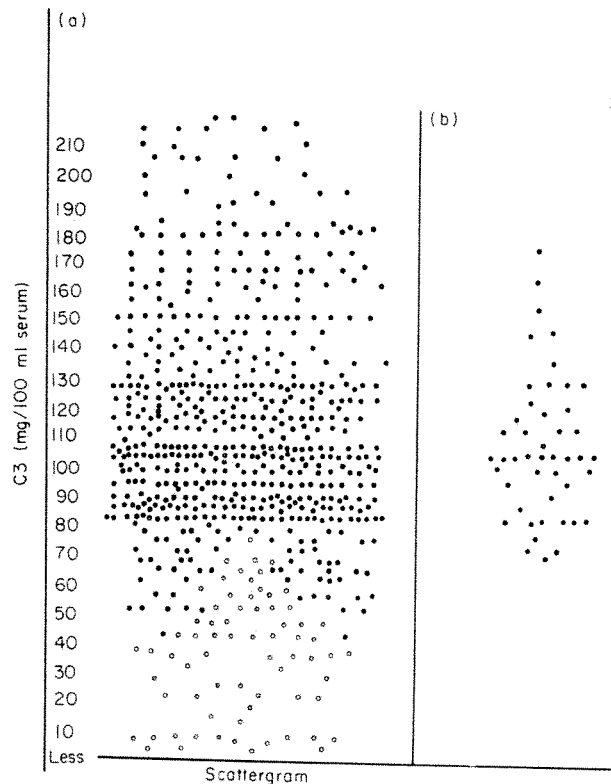


FIGURE 1. Scattergram illustrating C3 (beta A globulin) in (a) impetigo patients with and without nephritis, and (b) apparently healthy children.

(●) Impetigo patients and healthy children with normal urine; (○) impetigo patients with albumin, RBCs and casts in urine.

Urinalysis

Proteinuria, graded from a trace to 4+, was observed in 351 patients. Microscopic examination showed that 70 patients (19.3%) had more than 20 RBCs per high power field and had cellular and/or granular casts. In 281 patients, there were RBCs, WBCs with or without bilharzia ova, but no casts.

Serum C₃ level determination

Haemolysis caused difficulty in estimating the serum C₃ level in 4 healthy children and in 6 patients with impetigo. The mean level of serum C₃ in normal children was 108 ± 22.6 mg/100 ml, while in patients it was 106 ± 42.6 mg/100 ml. Individual serum C₃ levels are shown in Fig. 1. Most patients with a low serum C₃ level had nephritic changes, confirmed by urinalysis (Table 2).

There was a correlation between the serum C₃ level and the duration of disease. Thus, patients with C₃ levels between 0 and 70 mg/100 ml had a mean lesion duration of 22.2 days, for those with levels between 71 and 170 mg/100 ml the figure was 34.5 days and for those with levels between 171 and 200 mg/100 ml it was 98 days.

TABLE 2. Correlation between serum C₃ level and urinalysis

C ₃ (mg/100 ml serum)	No. of patients	Patients with no urine changes		Patients with urine containing Alb., RBCs and casts	
		No.	%	No.	%
< 10	14	0	0	14	100
11-20	3	0	0	3	100
21-30	9	0	0	9	100
31-40	11	0	0	11	100
41-50	16	2	12.5	14	87.5
51-60	25	14	56.0	11	44.0
61-70	30	23	76.6	7	23.3
71-80	26	25	96.1	1	3.9
81-90	94	94	100	0	0
91-100	59	59	100	0	0
101-110	69	69	100	0	0
111-120	56	56	100	0	0
121-130	64	64	100	0	0
131-140	28	28	100	0	0
141-150	31	31	100	0	0
151-160	10	10	100	0	0
161-170	22	22	100	0	0
171-180	23	23	100	0	0
181-190	8	8	100	0	0
191-200	8	8	100	0	0
201-210	11	11	100	0	0
211-220	4	4	100	0	0

Seasonal prevalence

The infectivity rate of the impetigo was high in summer and autumn but low in spring. There was more post-impetigo nephritis in summer (Table 3).

TABLE 3. Seasonal variation in impetigo and post-impetigo nephritis

Season	Patients		Patients with low C ₃ and U.N.*		
	No.	%	No.	%†	% of total
Summer	203	32.2	36	18	5.7
Autumn	188	29.9	12	6.4	1.8
Winter	161	25.7	15	9.4	2.4
Spring	75	12.1	7	9.3	1.1

* U.N., urine nephritis (contained albumin, RBCs, blood and granular casts).

† Percentage of patients in whom C₃ level was estimated.

Recovery of *Streptococcus pyogenes*

Beta-haemolytic streptococcus groups A, C and G were isolated from skin lesions of 573 patients (Table 4), group A (*Streptococcus pyogenes*) predominating.

TABLE 4. Groups of beta haemolytic streptococci* isolated from skin lesions

No. of patients	No. +ve HS†	No. of patients with HS*	+ve HS culture		No. of patients with single group	Mixed infect.	Groups of HS
			No.	%			
627	597	573	532	89.1	508	18	Group A
			46	7.7	28		Group G
			19	3.2	16		Group C

* HS, beta haemolytic streptococci.

† The number of +ve HS is more than number of patients because some patients had mixed infection even with group A streptococci.

Table 5 shows that 41% of patients were carrying *Streptococcus pyogenes* strains in the nose and/or throat, though few harboured them in the upper respiratory tract in the absence of skin lesions.

Streptococci of 16 serotypes were isolated from skin lesions, with predominance of T₃/B₃₂₆₄, T₁₃/B₃₂₆₄, T_{8/25}/Imp 19, T₁₄/49, T₁₁/27 and T₁₂ (Table 6). Three patients were infected with more than one type. The predominant serotypes isolated from patients with post-impetigo glomerulonephritis were T₄, T₁₄/49, T_{8/25}/Imp 19 and T₁₁. Two patients were infected with more than one type.

Typing showed that both T₄ and T₁₄/49 strains isolated from impetigo were M type 60 and M type 49 respectively, whether or not nephritis was present. Nineteen strains of T_{8/25}/Imp 19 pattern were type 55 and 11 of the patients developed nephritis. The rest of the strains could not be typed. The similarity between the serotypes isolated from the nose and from the skin lesions was greater than between those isolated from the throat and from the skin lesions (Table 7).

TABLE 5. Sites from which group A streptococci were recovered

No. of patients	<i>Strept. pyogenes</i> isolated	Patients with streptococci				Skin lesions with <i>Strept. pyogenes</i>						Skin lesions without streptococci							
		Skin lesion only		Skin lesion and throat		Skin lesion and nose		Skin lesion, nose and throat		Skin lesion		Throat		Nose		Throat and nose			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
627	532	529	84.4	27	44.0	93	14.9	91	14.4	72	11.5	0	0	14	2.2	11	1.8	4	9.7

TABLE 6. Streptococcal serotypes recovered from impetigo with or without glomerulonephritis

Types (T-protein)	No. of impetigo patients	No. with post- impetigo nephritis	No. of types	Types in uncompl. impetigo		Types in post- impetigo nephritis		No. of M. types	Types M. protein
				No.	%	No.	%		
I			9	5	55.5	4	44.4		
3/B			56	51	91.1	5	8.9		
3264									
13/B			42	41	97.6	1	2.4		
3264									
2			8	8	100	0			
4			18	5	27.8	13	72.2	13	60
6	627	70*	1	1	100	0	0		
28			27	27	100	0	0		
5			28	28	100	0	0		
11/27/44			33	25	75.8	8	24.2		
12/44			75	73	97.3	2	2.7		
8/25/Imp19			58	45	77.6	13	22.4	11	55
14/49			53	31	58.5	22	41.5	22	49
9			24	23	95.9	1	4.1		
18			3	3	100	0	0		
19			4	4	100	0	0		
23			1	1	100	0	0		
Non-typable stains			92	92	100	0	0		

* Patients with decrease serum C3 and haematuria, proteinuria and casts in urine.

One hundred houseflies examined yielded 18 strains of *S. pyogenes*, the T serotypes being as follows (number of isolates in parentheses): T3/B3264 (1), T2 (2), T4 (2), T28 (1), T5 (1), T11/27 (2), T8/25/Imp 19 (1), T14/49 (2), T9 (1), T23 (1), NT (4).

ASO response

The ASO titre was low in 57% of cases of impetigo. In post-impetigo nephritis the response was higher (Table 8). It was found that 62% of patients with high ASO titres had streptococci in skin lesions only (the upper respiratory tract having none).

TABLE 7. Types of streptococci recovered from the nose and throat in comparison to those of lesions

Condition	Nose			Throat			Throat and nose		
	No.	% Strep	% Total	No.	% Strep	% Total	No.	% Strep	% Total
Similar types to that of skin lesion	65	71.4	10.4	45	48.4	7.2	45	62.5	7.2
Different types from that of skin lesion	26	28.6	4.1	48	51.6	7.7	27	37.5	4.3

TABLE 8. Comparison of ASO titres in patients with complicated and uncomplicated impetigo

ASO titre (T.U.)	No. of patients	Patients with post-impetigo nephritis			Patients with uncomplicated impetigo		
		No.	%	% of total	No.	%	% of total
< 150	355	11	2	1.7	355	97	55.4
150-400	233	35	15	5.6	198	85	31.8
> 400	33	24	72.7	3.8	9	27.2	1.2

DISCUSSION

In studies carried out in the Red Lake Indian Reservation (Ferrieri *et al.*, 1972) and in Trinidad (Bassett, 1972) it was found that skin lesions (impetigo contagiosa) occurred mainly on the legs. Bassett (1967) suggested that the non-biting *Hippelates* flies, which often congregate on patient's legs, facilitate the spread of streptococci. The situation was very different in Egypt, where most of the patients had facial lesions, the legs usually being unaffected. Two factors may be implicated: (a) 11% of people harbour group A streptococci in the normal flora of their retroaural skin (El Tayeb & Nasr, 1975); and (b) house-flies are commonly found in rural areas and places where hygiene is poor, and on the children here, they are seen mainly on the face, acting, possibly, as a passive vector of *S. pyogenes*.

In the present study it would have been misleading to depend on haematuria and oedema for the diagnosis of post-impetigo nephritis for two reasons; firstly, urinary bilharziasis is endemic in Egypt where it is the main cause of haematuria; secondly, facial oedema is not a valid clinical sign of nephritis when, as is often the case, the original skin lesion is facial. Consequently, the serum C3 level alone offered a pathognomonic confirmatory diagnostic test in our cases, and post-impetigo nephritis was in fact diagnosed on the strength of a falling serum C3 level. However, 5% of these patients had a normal urine and were therefore considered to be cured cases of nephritis. There seemed to be a relationship between a rise in the serum C3 level and a prolonged exposure to infection, due, possibly, to the latter stimulating hepatic synthesis of complement components (Pont & Good, 1967).

Both impetigo contagiosa and nephritis were more frequent in summer, due possibly to the increased fly population in the hot weather, enhancing the transmission of streptococci.

Despite the fact that *Streptococcus pyogenes* strains were found to be the main causative agent, acute glomerulonephritis did not appear in epidemic form. Of the T-typable strains, 90% belonged to nine types, each of which appeared with more or less the same frequency; there was no predominance of any one specific type such as occurred in Trinidad (Parker *et al.*, 1968), and Red Lake (Anthony *et al.*, 1969; Kaplan *et al.*, 1970). This may explain why nephritis occurs only as sporadic cases in Egypt.

Type M 60 does not have a high infectivity, but it proved to be highly nephritogenic, while type M 49 was only moderately so. The situation with type M 55 was very surprising since it was isolated from only 19 patients with impetigo, and yet of whom 11 developed nephritis. In a previous study in Egypt (El Tayeb *et al.*, 1968) on sporadic cases of nephritis this type was isolated from throats and formed 47% of typable strains, whereas in Trinidad it was the main causative agent of epidemics of post-impetigo nephritis (Parker *et al.*, 1968).

In spite of the high infectivity rate of T12 it is notable that this strain rarely caused nephritis in Egypt, although it was thought to be nephritogenic type in the last decade (Kodoma *et al.*, 1958;

Rammelkamp, 1964). However T12 strains need not be M12 and it is the M antigen which is of importance.

El Zawahry *et al.* (1972) reported a lower frequency of *S. pyogenes* strains in lesions than we did (30% versus 84%). El Kholy *et al.* (1973), however, isolated *S. pyogenes* from 77% of cases, but 72% of the lesions were secondarily infected traumatic abrasions or cuts.

Nasal carriers of streptococci outnumbered throat carriers. This might be due to spread of streptococci from lesions on the skin, to the nose, and thence to the throat (Ferrieri *et al.*, 1972).

ASO responses were feeble in our patients; this is in keeping with the findings of Anthony *et al.* (1967), Dillon & Reeves (1969) and El Kholy *et al.* (1973). This mild response has been attributed to the presence of skin lipids (Ricketts *et al.*, 1951; Kaplan & Wannamaker, 1972). The rise in the ASO titre in post-impetigo nephritis may be due to factors peculiar to the individual including hypersensitivity or disturbed immunological reactions; nephritis is presumably an immune complex disease (Derrick, Reeves & Dillon, 1970).

REFERENCES

- ANTHONY, B.F., PERLMAN, L.V. & WANNAMAKER, L.W. (1967) Skin infection and acute glomerulonephritis in American Indian children. *Pediatrics (Springfield)*, **39**, 263.
- ANTHONY, B.F., KAPLAN, E.L., WANNAMAKER, L.W., BRIESE, F.W. & CHAPMAN, S.S. (1969) Attack rates of acute glomerulonephritis after type 49 streptococcal infection of the skin and of respiratory tract. *Journal of Clinical Investigations*, **48**, 1697.
- BASSETT, D.C.J. (1967) Hippelates flies and acute glomerulonephritis. *Lancet*, **i**, 503.
- BASSETT, D.C.J. (1972) Streptococcal pyoderma and acute glomerulonephritis in Trinidad. *British Journal of Dermatology*, **86**, Suppl. 1, 55.
- DERRICK, C.W., REEVES, M.S. & DILLON, H.C. JR. (1970) Complement in overt and asymptomatic nephritis after skin infection. *Journal of Clinical Investigations*, **49**, 1179.
- DILLON, H.C. & REEVES, M.S. (1969) Streptococcal antibody titres in skin infection and acute glomerulonephritis. *Pediatric Research*, **3**, 362.
- EL-KHOLY, A.M., SOROUR, A.H., ROTTA, J. & GUIRGUIS, N. (1973) Group A beta hemolytic streptococci in skin lesions among Egyptian school children population. *Journal of Hygiene Epidemiology, Microbiology and Immunology*, **17**, 316.
- EL-TAYEB, S.H., MAXTED, W.R. & PARKER, M.T. (1968) Types of *Streptococcus pyogenes* from sporadic cases of nephritis. Unpublished data.
- EL-TAYEB, S.H.M. & NASR, E.M.M. (1975) *Streptococcus pyogenes* as a normal flora of throat, nose and skin in asymptomatic school age children. *Journal of the Egyptian Public Health Association*, **5**, 295.
- EL-ZAWAHRY, M., ABDEL AZIZ, A. & SOLIMAN, M. (1972) The aetiology of impetigo contagiosa. *British Journal of Dermatology*, **87**, 420.
- FERRIERI, P., DAJANI, A.S. & WANNAMAKER, L.W. (1972) Natural history of impetigo. 1. Site sequence of acquisition and familial patterns of spread of cutaneous streptococci. *Journal of Clinical Investigation*, **51**, 2851.
- GRIFFITH, F. (1934) The serological classification of *Streptococcus pyogenes*. *Journal of Hygiene (Cambridge)*, **24**, 542.
- KAPLAN, E.L., ANTHONY, B.F., CHAPMAN, S.S. & WANNAMAKER, L.W. (1970) Epidemic acute glomerulonephritis associated with type 49 streptococcal pyoderma. 1. Clinical and laboratory finding. *American Journal of Medicine*, **48**, 9.
- KAPLAN, E.L. & WANNAMAKER, L.W. (1972) Suppression of host response to streptolysin O by skin lipids and its clinical application. *Pediatric Research*, **7**, 147.
- KLEINMAN, H. (1954) Epidemic acute glomerulonephritis at Red Lake, Minnesota. *Medicine*, **37**, 479.
- KODOMA, T., MIYOMOTO, Y., KOTAKE, I. & OCHIAI, S. (1958) Epidemic nephritis in Japan. *Yokohama Medical Bulletin*, **9**, 105.
- LANCEFIELD, R.C. (1933) A serological differentiation of human and other groups of hemolytic streptococci. *Journal of Experimental Medicine*, **57**, 571.
- MARKHAM, N.P. & STENHOUSE, A.C. (1959) A bacteriological investigation of wound infections in Raroconga, Cook Islands. *Transactions of the Royal Society for Tropical Medicine and Hygiene*, **53**, 404.

- PARKER, M.T., BASSETT, D.C.J., MAXTED, W.R. & ARNEAUD, J.D. (1968) Acute glomerulonephritis in Trinidad: Serological typing of group A streptococci. *Journal of Hygiene (Cambridge)*, **66**, 657.
- PIKE, R.M. (1944) An enrichment broth for isolating hemolytic streptococci from throat swabs. *Proceedings of the Society for Experimental Biology and Medicine*, **57**, 186.
- PONT, B.H. & GOOD, R.A. (1967) Synthesis of complement, inflammation and immunologic injuries. In: *Principles of Modern Immunobiology*, pp. 150 and 173.
- RAMMELKAMP, C.H. JR. (1964) *Concepts of Pathogenesis of Glomerulonephritis* (ed by J. Uhr), pp. 289-300.
- RANTZ, L.A. & RANDALL, E. (1945) Modification of technique for determination of antistreptolysin titre. *Proceedings of the Society for Experimental Biology and Medicine*, **59**, 22.
- RICKETTS, C.R., SQUIRE, J.R. & TOPLEY, E. (1951) Human skin lipids with particular reference to the self sterilizing power of skin. *Clinical Science*, **10**, 89.
- SWIFT, H.F., WILSON, A.T. & LANCEFIELD, R.C. (1943) Typing group A streptococci by M-precipitin reactions in capillary pipettes. *Journal of Experimental Medicine*, **78**, 127.
- WANNAMAKER, L.W. (1970) Differences between streptococcal infections of throat and of skin. *New England Journal of Medicine*, **282**, 78.

Myocardial infarction, androgen and the skin

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SUMMARY

Various indices of masculinity were compared in 48 men who had recovered from myocardial infarction and in their age-matched controls. We found little evidence to support the idea that myocardial infarction is related to increased androgenic stimulation. The patients with myocardial infarction had no increase in plasma testosterone, muscle thickness, sebum excretion rate, maximal sweat secretion rate, male pattern alopecia or density of terminal body hair, but as a group they had a slight increase in skin and bone thickness compared with the controls.

Myocardial infarction is commoner in men than women and this suggests that masculinity predisposes to atheroma formation. One study has shown that male-pattern alopecia (M.P.A.) is associated with an increased risk of myocardial infarction compared with age-matched controls (Cotton *et al.*, 1972), and androgenic stimulation is a pre-requisite to the development of M.P.A. (Hamilton, 1942). Other factors associated with masculinity might therefore also be linked with a tendency to atheroma. Sebum excretion rate (S.E.R.), sweat secretion rate (S.S.R.), skin thickness and the density of terminal body hair are increased by androgenic stimulation (Pochi & Strauss, 1963; Strauss & Pochi, 1963; Burton *et al.*, 1972; Black, 1969; Shuster, Black & Bottoms, 1970; Black, Shuster & Bottoms, 1970), and so we have measured these, together with muscle thickness, bone thickness and plasma testosterone, in men with myocardial infarction and compared them with control measurements.

METHODS

We studied 48 men aged 35–64 years (mean $51.4 \pm \text{s.d. } 6.8$) who had had one myocardial infarction (confirmed by ECG and enzyme changes) managed in hospital in the previous 4 years and who had made a complete recovery and resumed normal activities. We compared these men with 48 age-matched control men (mean age $51.4 \pm \text{s.d. } 6.8$) from the same hospital population who had had a surgical operation for a benign condition such as hernia or appendicitis in the same period, but we excluded patients with conditions such as prostatic hypertrophy which might be related to an abnormal endocrine state. In both groups we excluded men with hypertension (B.P. exceeding 140/90 mmHg)

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or other serious disease such as diabetes mellitus, and none had a history of familial hypercholesterolaemia. None of the men we studied was taking drugs such as phenothiazines, digoxin, barbiturates or endocrine preparations which might have affected our measurements.

A full history was taken from each subject, including details of his present and previous occupations and the degree of physical exertion involved in his work, which was graded 1 (sedentary) to 4 (heavy labouring). The family history of hypertension, angina, myocardial infarction, cerebrovascular accident, intermittent claudication and sudden death was recorded for the mother, father and brothers of each subject. We recorded details of personal and family history of acne vulgaris and prostatic hypertrophy. Each subject was shown a diagram of 5 different grades of severity of M.P.A. (Fig. 1) and asked which grade best fitted the hair pattern of his father and brothers at the time of the study (or at the time of their death). One author (M.B.) used the same diagram to grade the M.P.A. of each subject.

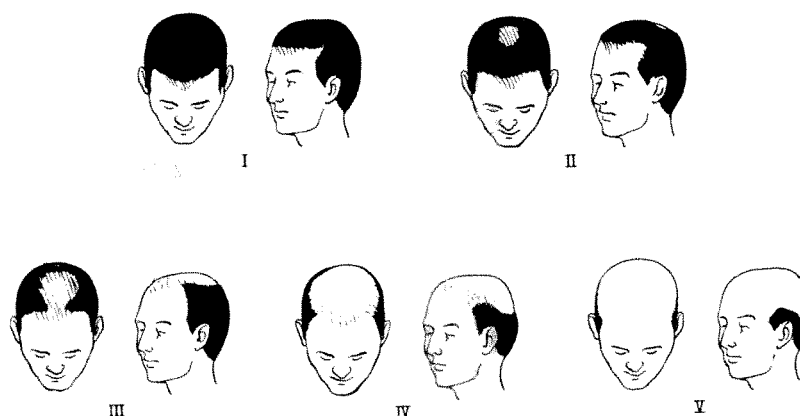


FIGURE 1. Diagrams used to grade the severity of male-pattern alopecia (modified from Rook *et al.*, 1972).

A full examination was performed including measurement of height, weight and blood pressure. Each subject was requested to lie flat on a couch and relax for 5 min. The supine blood pressure was measured at least once at the end of this rest period, and again at the end of the examination. The lowest values were recorded, and any subject with a systolic pressure above 140 mmHg or a diastolic pressure above 90 mmHg was excluded. Bone, fat and muscle thickness midway down the right upper arm were measured by a radiographic method (Tanner, 1964). The arm was positioned so that the two epicondyles were superimposed on a lateral view and the measurement of humerus and muscle thickness was made with a ruler directly from the film. Forearm skin thickness was measured radiographically from a projected image of the film using a modification of Black's method (Black, 1969; Jeans, 1977).

The S.E.R. from forehead skin was measured gravimetrically (Cunliffe & Shuster, 1969) and the maximal S.S.R. from the flexor surface of the forearm was measured following the iontophoresis of 0.2% pilocarpine into the skin (Johnson & Shuster, 1969). We used a 15 min collection period, and the mean value from both forearms was obtained.

The density of terminal hair on the chest, forearm, lower leg, dorsum of hand and tragus of ear was estimated visually in each area and graded from 1 (absent) to 4 (profuse) by one author (M.B.), using reference photographs to ensure that his criteria remained constant. The corneal arcus was graded 1–3 according to whether it was absent, partial or complete.

A single estimation of plasma testosterone was made in each subject at 9 a.m. using a radio-immuno-assay (Kinouchi, Pages & Horton, 1973).

The measurements described above were made in the same order and at approximately the same time of day in each subject, and infarct patients and controls were tested together. The reproducibility of each method was tested and found to be satisfactory.

RESULTS

The two groups did not differ significantly as regards their personal and family history of acne vulgaris and prostatism, nor with regard to their height, weight, marital status, mean social class (Registrar General's classification by occupation) and the degree of physical exertion involved in their work. The

TABLE 1. Prevalence of the various grades of male-pattern alopecia in men with myocardial infarction and age-matched controls

M.P.A. Grade	1	2	3	4	5
No. of subjects:					
Patients	20	7	14	7	0
Controls	13	8	20	7	0

TABLE 2. Indices of androgen status in 48 men with myocardial infarction and age-matched controls

	Infarction group		Controls		Significance (Wilcoxon's test)
	Mean	s.e.	Mean	s.e.	
Plasma testosterone (nmol/l)	17.5	0.9	17.5	1.2	N.S.
Sebum excretion rate ($\mu\text{g}/\text{cm}^2/\text{min}$)	0.90	0.06	0.97	0.06	N.S.
Maximal sweat secretion rate ($\text{g}/\text{m}^2/\text{min}$)	5.28	0.34	6.30	0.37	$P < 0.01$
Muscle thickness (cm)	7.70	0.11	7.74	0.11	N.S.
Bone thickness (cm)	2.35	0.03	2.28	0.03	$P < 0.01$
Skin thickness (mm)	1.35	0.04	1.26	0.03	$P < 0.05$

mean systolic blood pressure was $127.4 \pm \text{s.e. } 1.2$ mmHg in the control group and $127.0 \pm \text{s.e. } 1.2$ mmHg in the myocardial infarction group. The mean diastolic blood pressure was $81.9 \pm \text{s.e. } 0.9$ mmHg in the control group and $81.6 \pm \text{s.e. } 0.9$ mmHg in the myocardial infarction group.

The prevalence of M.P.A. in the two groups of men did not differ significantly (Table 1) even when the data for the more severe grades (3-5) were pooled. Neither did we find any evidence of an increase in baldness in the fathers and brothers of the myocardial infarction patients. 42% of 106 male relatives of the infarction group were judged to have M.P.A. of grades, 3-5, compared with 33% of 120 male relatives of the control group, but this increase was not significant. The density of terminal hair on the chest, forearm, lower leg, dorsum of hand and tragus of ear did not differ significantly in the two groups of men.

A family history of cardiovascular disease and cerebrovascular disease were both more common in the men with myocardial infarction. Twenty-nine per cent of 150 relatives of the infarction group had suffered from either hypertension, angina or myocardial infarction compared with 18% of 165 relatives of the control group ($\chi^2=5.4$, $P<0.02$). Thirteen percent of the relatives of the infarction

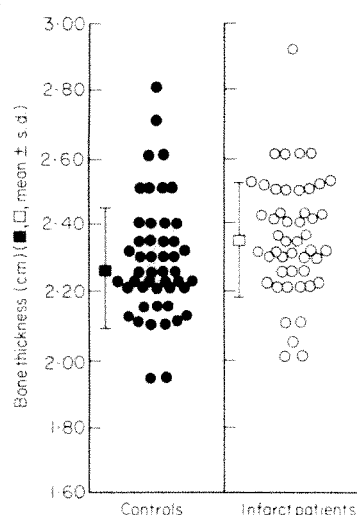


Fig. 2.

FIGURE 2. Right mid-humeral thickness in myocardial infarct patients and controls.

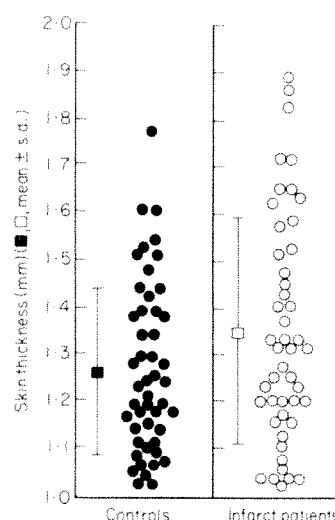


Fig. 3.

FIGURE 3. Forearm skin thickness in myocardial infarct patients and controls.

group had suffered from a stroke compared with 5% of the relatives of the controls ($\chi^2=5.6$, $P<0.02$).

Corneal arcus also occurred more commonly in the men with myocardial infarction. Forty-four percent of this group had at least a partial corneal arcus compared with 25% of the control subjects. A complete corneal ring was seen in 9 infarction patients but in only 3 control patients ($\chi^2=4.17$, $P<0.05$).

The results of the tests of 'androgen status' are shown in Table 2. No significant difference was found in the mean plasma testosterone, muscle thickness or S.E.R. in the 2 groups, but the maximal sweat secretion rate was slightly decreased in the infarction group. The mean skin thickness and bone thickness, by contrast, showed a small but significant increase in the infarction group (Figs 2 and 3). The mean ponderal index ($\text{Weight} \div \text{Height}^2 \times 100$) for the infarction patients ($25.3 \pm \text{s.e. } 0.39$) was not significantly greater than the value for the controls ($25.0 \pm \text{s.e. } 0.37$).

DISCUSSION

The literature relating androgenic stimulation and myocardial infarction is sparse and conflicting. An American study showed that myocardial infarct patients under 40 were morphologically more mascu-

line than controls (Gertler, Garn & White, 1951) but a Russian study found that men with ischaemic heart disease had a decreased urinary excretion of androgens (Baratz & Yordanidi, 1973). Phillips found that young men (aged 32–42) with myocardial infarction had normal serum androgens but significantly increased oestrogens, and he found that signs of feminisation, particularly with regard to beard growth, correlated with myocardial infarction (Phillips, 1976). Our patients in an older age range (35–65) also had normal levels of plasma testosterone, but neither study excludes the possibility that the patients had been less active sexually following their infarction, and there is some evidence that sexual activity may increase testosterone production (Ismail & Harkness, 1967). Myocardial infarction could also relate to androgenic stimulation as a result of an increased androgenic inter-conversion or enhanced end-organ sensitivity to normal androgen levels, but our study provides little support for this idea. Men with myocardial infarction had no increase in sebum excretion rate, muscle thickness, body hair or male pattern alopecia, and their maximal sweating rate was slightly decreased compared with controls. This decrease in sweating ability may have resulted from a tendency for myocardial infarction patients to avoid heavy muscular exertion, since the maximal sweat rate increases with strenuous physical training. This might also explain our failure to find an increase in muscle thickness. We found a slight but significant increase in bone and skin thickness, and this might relate to the association between myocardial infarction and a mesomorphic somatotype (Levine & Brown, 1929; Spain, Bradess & Huss, 1953; Shanoff *et al.*, 1961) which may itself reflect 'androgen status'. There was no increase in ponderal index in our infarction patients, and it is possible that the increased skin and bone thickness was a sign of increased androgenic stimulation unrelated to mesomorphy.

Our failure to confirm the finding of Cotton *et al.* (1972) that baldness is a good discriminator between myocardial infarction patients and controls, probably represents a difference in selection. Their study did not exclude hypertensive patients, and yet their infarction group had a lower incidence of family history of hypertension than their control group. Their choice of blood donors as controls is also open to criticism (Leading article, *B.M.J.*, 1972), and they analyzed their results by Student's *t*-test, which is not strictly applicable to discrete data with a skew distribution.

We conclude that men with myocardial infarction show little evidence of increased masculinity compared with age-matched controls.

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REFERENCES

- BARATZ, S.S. & YORDANIDI, S.A. (1973) Androgen excretion in males suffering from angina pectoris, and changes in lipoprotein metabolism and blood coagulation in patients with androgen deficiency. *Kardiologiya*, **13**, 74.
- BLACK, M.M. (1969) A modified radiographic method for measuring skin thickness. *British Journal of Dermatology*, **81**, 661.
- BLACK, M.M., SHUSTER, S. & BOTTOMS, E. (1970) Osteoporosis, skin collagen and androgen. *British Medical Journal*, **iv**, 773.
- BURTON, J.L., JOHNSON, C., LIBMAN, L. & SHUSTER, S. (1972) Skin virilism in women with hirsutism. *Journal of Endocrinology*, **53**, 349.

- COTTON, S.G., NIXON, J.M., CARPENTER, R.G. & EVANS, D.W. (1972) Factors discriminating men with coronary heart disease from healthy controls. *British Heart Journal*, **34**, 458.
- CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697.
- GERTLER, M.M., GARN, S.M. & WHITE, P.D. (1951) Young candidates for coronary heart disease. *Journal of the American Medical Association*, **147**, 621.
- HAMILTON, J.B. (1942) Male hormone stimulation is prerequisite and an incitant in common baldness. *American Journal of Anatomy*, **71**, 451.
- ISMAIL, A.A.A. & HARKNESS, R.A. (1967) Urinary testosterone excretion in men in normal and pathological conditions. *Acta Endocrinologica*, **56**, 469.
- JEANS, W.D. (1977) M.D. Thesis, in preparation.
- KINOCHI, T., PAGES, L. & HORTON, R. (1973) A specific radio-immunoassay for testosterone in peripheral plasma. *Journal of Laboratory Medicine*, **82**, 309.
- LEADING ARTICLE (1972) Predicting coronary artery disease. *British Medical Journal*, **7**, 3.
- LEVINE, S.A. & BROWN, L.L. (1929) Coronary thrombosis; its various clinical features. *Medicine*, **8**, 257.
- PHILLIPS, G.B. (1976) Evidence for hyperoestrogenaemia as a risk factor for myocardial infarction in men. *Lancet*, **ii**, 14.
- POCHI, P.E. & STRAUSS, J.S. (1963) Sebaceous gland function before and after bilateral orchiectomy. *Archives of Dermatology*, **88**, 729.
- ROOK, A., WILKINSON, D.S. & EBLING, F.J.G. (1972) *Textbook of Dermatology*, 2nd ed, Vol. 2, p.1588. Blackwell Scientific Publications, Oxford.
- SHANOFF, H.M., LITTLE, A., MURPHY, E.A. & RYKERT, H.E. (1961) Studies of male survivors of myocardial infarction due to 'essential' atherosclerosis. I. Characteristics of the patients. *Canadian Medical Association Journal*, **84**, 519.
- SHUSTER, S., BLACK, M.M. & BOTTOMS, E. (1970) Skin collagen and thickness in women with hirsuties. *British Medical Journal*, **iv**, 772.
- SHUSTER, S. & JOHNSON, C. (1969) The abnormality of sweat duct function in psoriasis. *British Journal of Dermatology*, **81**, 846.
- SPAIN, D.M., BRADESS, V.A. & HUSS, G. (1953) Observations on atherosclerosis of the coronary arteries—males under the age of 46. Necropsy study with special reference to somatotypes. *Annals of Internal Medicine*, **38**, 254.
- STRAUSS, J.S. & POCHI, P.E. (1963) The human sebaceous gland. Its regulation by steroidal hormones and its use as an end-organ for assaying androgenicity *in vivo*. *Recent Progress in Hormone Research*, **19**, 385.
- TANNER, J.M. (1964) *The Physique of the Olympic Athlete*. Allen and Unwin, London.

Gluten-free diet in dermatitis herpetiformis

II. MORPHOLOGICAL AND IMMUNOLOGICAL FINDINGS IN THE SKIN AND SMALL INTESTINE OF 12 PATIENTS AND MATCHED CONTROLS

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SUMMARY

Twelve patients with dermatitis herpetiformis whose skin condition responded to a gluten-free diet (GFD) were re-examined after diet treatment. The findings were compared with those in matched patients on a normal diet.

Jejunal histology revealed morphological improvement in every patient on a GFD whereas all patients on a normal diet continued to have villous atrophy. Intra-epithelial lymphocyte counts were normal in 8 patients on a GFD in contrast to one on a normal diet. Immunofluorescence examination of the jejunal mucosa revealed that the numbers of cells containing IgA and IgM were increased significantly in the normal diet group. The figures were lower in the GFD group but these also exceeded the values in the controls.

IgA deposits were found in the uninvolved skin of every patient irrespective of the diet treatment, but the fluorescence seemed to be less intense in patients on a GFD. A clear difference was found in the occurrence of C3 deposits in the uninvolved skin. Three patients on a GFD had C3 deposits; two of these did not follow a strict diet. However C3 was found in 8 patients on a normal diet.

Circulating dietary and auto-antibodies were found in two patients on a GFD and in 9 on a normal diet. Serum immunoglobulin (IgG, IgA, IgM) and complement (C3, C4) levels were within normal limits in both patient groups.

There is considerable evidence to suggest that immunological factors are of great importance in dermatitis herpetiformis (DH) and in coeliac disease (CD). A number of immunological abnormalities have been described in both diseases. These include abnormal IgA and IgM levels in the serum and in the intestinal juice (Fraser, Dick & Crichton, 1969; Douglas, Crabbe & Hobbs, 1970; McClelland *et al.*, 1972), and the presence of circulating antibodies and immune complexes (Seah *et al.*, 1973; Kumar *et al.*, 1976; Mohammed *et al.*, 1976). The presence of IgA deposits in the skin of patients with

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DH seems to be the only basic difference between DH and CD, and this may account for the absence of skin lesions in CD (van der Meer, 1969; Seah *et al.*, 1972). These IgA deposits are of great importance in the diagnosis of DH because they seem to be present in almost every patient (Seah & Fry, 1975).

Gluten sensitivity is a common underlying factor in DH and CD. There is general agreement that gluten withdrawal in DH improves the intestinal lesion which is found in about 2/3 of patients (Marks, Shuster & Watson, 1966; Fry *et al.*, 1968). Different opinions have been expressed concerning the effect of gluten-free diet (GFD) treatment on the skin in patients with DH, but recent studies have shown that the rash also responds to this treatment (Fry *et al.*, 1973; Heading *et al.*, 1976; Reunala *et al.*, 1977).

In this study the effect of GFD treatment on morphological and immunological findings in the skin and small intestine was examined in 12 patients with DH whose skin condition improved with a GFD. The results were compared with the findings in 12 matched DH patients on a normal diet.

PATIENTS AND METHODS

Patients

Twelve patients on a GFD were selected for this study from 81 patients with DH treated with this diet (Reunala *et al.*, 1977). All 12 patients had jejunal villous atrophy at the beginning of the GFD. During the diet a decrease of at least 25% in daily dapsone requirement was observed in all patients indicating a clinical response to GFD treatment. A control group consisted of 12 patients with DH on a normal diet. Each control patient was matched with one patient on a GFD for the severity of the intestinal lesion, for the daily dapsone requirement at the beginning of the diet and for the duration of diet treatment. Attention was also paid to age, sex and duration of dapsone treatment before the diet. After selection there were 8 male and 3 female pairs and one male-female pair.

All 24 patients were re-examined as in-patients. When the patients were invited to attend the re-examination they were once again asked to try to reduce their dapsone dose to the minimum required to control the skin symptoms, i.e. keep them at a tolerable level. During the re-examination the dapsone requirement and the presence of the rash were recorded and the strictness of the GFD was evaluated in a personal interview.

Methods

Jejunal biopsies were performed as described earlier (Reunala *et al.*, 1976a). The biopsy specimens taken before the diet were re-stained with haematoxylin and eosin. The specimens taken at the re-examination were divided for ordinary histology and for immunofluorescent (IF) study. The heights of the villi were measured from histological sections as described elsewhere (Reunala *et al.*, 1977). The total number of intraepithelial lymphocytes per 500 epithelial cell nuclei was counted from 7 μ m thick sections under $\times 1250$ magnification (Ferguson & Murray, 1971). A villous height of over 300 μ m and an intraepithelial lymphocyte count of below 40/100 epithelial cells were considered normal.

Jejunal biopsy specimens for IF study were fixed in 2.5% phosphate buffered formaldehyde, pH 7.2, for 2 h, transferred to 30% sucrose and left there overnight (Savilahti, 1972). Skin was taken by 5 mm punch biopsy from uninvolved skin on the flexor surface of the forearm. The fixed jejunal and unfixed skin specimens were embedded in Ames OCT compound and stored at -50°C . Tissue sections 5 μ m thick were cut in a cryostat at -20°C and were processed by the standard methods for direct IF (Beutner & Nisengard, 1973). The following commercial conjugates (Behringwerke) were used: antihuman IgA, batch no. 678B, molar fluorescein-to-protein ratio (F/P) 2.6, dilution (D) 1:20; antihuman IgM, batch no. 665D, F/P 2.4, D 1:20; antihuman IgG, batch no. 643A, F/P 2.0, D 1:40;

antihuman C3, batch no. 619S, F/P 2.0, D 1:20. The results were read with a Leitz Orthoplan fluorescent microscope with incident illumination, a xenon light source, two KP 490 excitation filters and a OG 510 barrier filter.

The intensity of the fluorescence in the papillary dermis of the uninvolved skin was graded by observing 4–5 tissue sections. Positively stained immunoglobulin (Ig)-containing cells of the jejunal mucosa were counted under $\times 800$ magnification using an eyepiece with a measurement grid. Cells were counted from an area of 0.2 mm^2 between the epithelium and muscularis mucosae (Savilahti, 1972). The control values of villous height, intraepithelial lymphocytes and Ig-containing cells were obtained from jejunal biopsy specimens of 12 patients with no evidence of gluten-sensitive enteropathy. Four of these patients had recurrent aphthous stomatitis, one pemphigoid, and the others various chronic gastrointestinal symptoms.

Epicutaneous tests were performed with 50, 25 and 12.5% potassium iodide (KI) in petrolatum. The results were read after 24 h.

The following routine laboratory tests were performed: Hb, WBC, ESR, serum transaminases, bilirubin and electrophoresis. Serum dapsone concentration was measured as described elsewhere (Reunala *et al.*, 1977). Serum immunoglobulins were quantified by single radial immunodiffusion using commercial plates (Tri-Partigen, Behringwerke). Antireticulin antibodies (ARA), gastric parietal cell antibodies (PCA) and antibodies to cow's milk and gluten were determined as described earlier (Reunala *et al.*, 1976b). Antinuclear antibodies (ANA) and serum complement levels were determined in the Laboratory of Clinical Immunology; ANA by the indirect IF method using mouse liver cryostat sections as a substrate and monovalent and polyvalent anti-Ig-conjugates, C3 and C4 levels using automatic fluoronephelometric immunoprecipitation assay (Technicon).

RESULTS

The clinical data of the two patient groups are given in Table 1. At the re-examination the mean daily

TABLE 1. Clinical data of the patients

Patients	Mean age years (range)	Mean duration of diet years (range)	Daily dapsone requirement (mg)	
			I	II
Gluten-free diet (n=12)	34.8 (20–55)	3.2 (1.0–6.8)	88 $\pm$ 31*	25 $\pm$ 34
Normal diet (n=12)	38.1 (22–60)	3.4 (1.0–7.3)	88 $\pm$ 58	77 $\pm$ 61

* Data are means $\pm$ s.d.; I—at the beginning of diet; II—at the re-examination.

dapsone requirement had decreased from 88 to 25 mg in the GFD group. Five patients in this group were able to manage without dapsone. In contrast, every patient in the normal diet group needed dapsone and there was only a slight change in the mean daily dapsone requirement.

Skin

The rash was milder in the GFD group (Table 2). Six patients on a GFD, 5 of whom were without dapsone, had no rash at all, in contrast to one patient in the normal diet group. IgA was found in the uninvolved skin of every patient irrespective of the diet treatment (Table 2). IgA deposits were granular and none of the patients had a linear pattern of IgA deposition. Weak IgM fluorescence was detect-

TABLE 2. Clinical picture of the rash and immunofluorescent (IF) findings in the uninvolved skin

Patients	Clinical picture				IF findings in the papillary dermis							
	Grade				IgA				C3			
	III*	II	I	0	++†	+	(+)	—	++	+	(+)	—
Gluten-free diet (n=12)	0	3	3	6	3	7	2	0	0	1	2	9
Normal diet (n=12)	1	7	3	1	7	4	1	0	2	6	0	4

* III=many active lesions; II=some active lesions and/or many crusts; I=only some crusts; 0=no lesions.

† ++ Signs indicate the intensity of the fluorescence.

ed in one patient in each group. No IgG deposits were found. C3 deposits were found in sites corresponding to those of IgA deposition. In the GFD group 3 patients had C3 deposits in contrast to 8 in the normal diet group. This difference is almost significant (Fisher's exact test, $P<0.05$).

The two groups had similar skin responses to KI in epicutaneous tests. Two patients in each group were negative to 12.5% test concentration, two on a GFD and one on a normal diet were negative to every concentration of KI. All the others had positive results.

Serum

There were only slight differences in the mean values of different laboratory tests between the two groups. The mean level of C3 was lower and the mean concentration of dapsone higher in patients on a normal diet but the differences were not statistically significant (Table 3). The following antibodies

TABLE 3. Laboratory data of the patients

Patients	Haemoglobin (g/100 ml)	Serum immunoglobulins			Serum complement		Serum dapsone (mg/100 ml)
		IgG	IgA	IgM	C3	C4	
Gluten-free diet (n=12)	13.8 ± 1.1	1410 ± 310*	240 ± 100	140 ± 60	122 ± 31	20 ± 6	0.08 ± 0.11
Normal diet (n=12)	14.2 ± 1.0	1430 ± 290	260 ± 100	140 ± 80	98 ± 35	20 ± 6	0.15 ± 0.11

* Data are means ± s.d. Normal limits (mg/100 ml): IgG, 800-1800; IgA, 90-450; IgM, 60-250; C3, 55-140; C4, 12-34.

were found in the serum of 9 patients on a normal diet: ANA in two patients (titre 1/10); ARA in 4 (titre 1/100); PCA in one (titre 1/100); antibodies to cow's milk in 4, two of whom also had antibodies to gluten. In the GFD group one patient had ANA and one antibodies to cow's milk.

Small intestine

The effect of GFD treatment on the intestinal lesion is shown in Figs 1 and 2. One patient on a GFD had mild villous atrophy and 4 had increased intraepithelial lymphocyte counts at the re-examination.

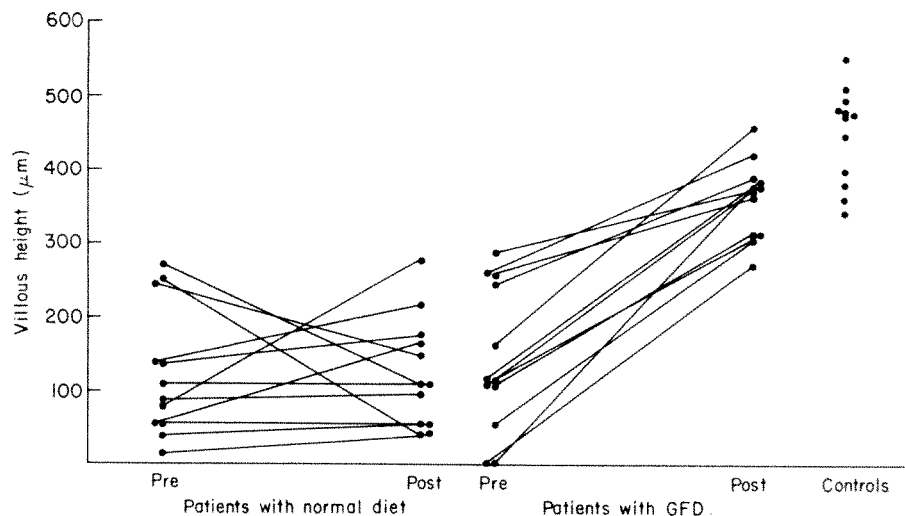


FIGURE 1. Jejunal morphology before and after treatment in 12 patients on a normal diet and in 12 patients on a gluten-free diet (GFD).

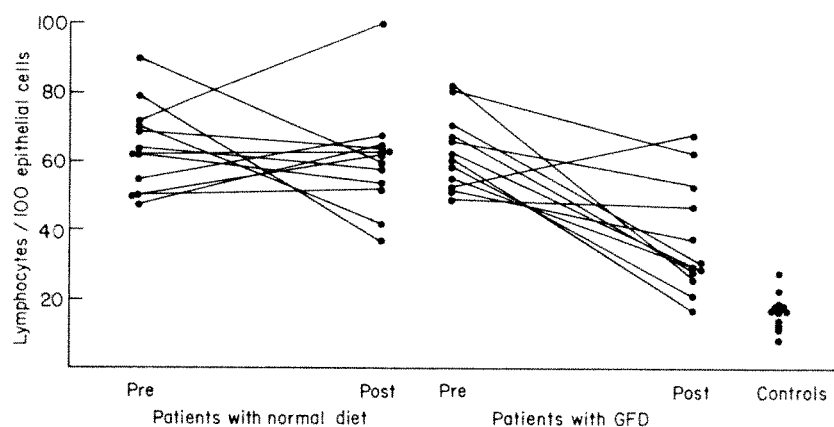


FIGURE 2. Intraepithelial lymphocytes in the jejunal mucosa before and after treatment in 12 patients on a normal diet and in 12 patients on a gluten-free diet (GFD).

In contrast, all patients on a normal diet continued to have villous atrophy and 11 of these had increased lymphocyte counts.

Before the diet treatment the mean villous height was $143 \pm 101 \mu\text{m}$ and the mean intraepithelial lymphocyte count/100 epithelial cells was 63 ± 11 in the GFD group. The corresponding figures were 124 ± 88 and 64 ± 13 in the normal diet group. At the re-examination the figures were 362 ± 53 and 38 ± 16 in the GFD group and 125 ± 75 and 60 ± 16 in the normal diet group. The differences are significant in the GFD group ($t = 6.7$, $P < 0.001$ for villous height; $t = 4.5$, $P < 0.001$ for lymphocyte count). Although mucosal improvement was clear in the GFD group the mean villous height and intraepithelial lymphocyte count still remained far from the values in the controls (mean villous height 451 ± 65 , mean lymphocyte count 17 ± 5). The differences are significant ($t = 3.7$, $P < 0.01$ for villous height; $t = 4.3$, $P < 0.001$ for lymphocyte count).

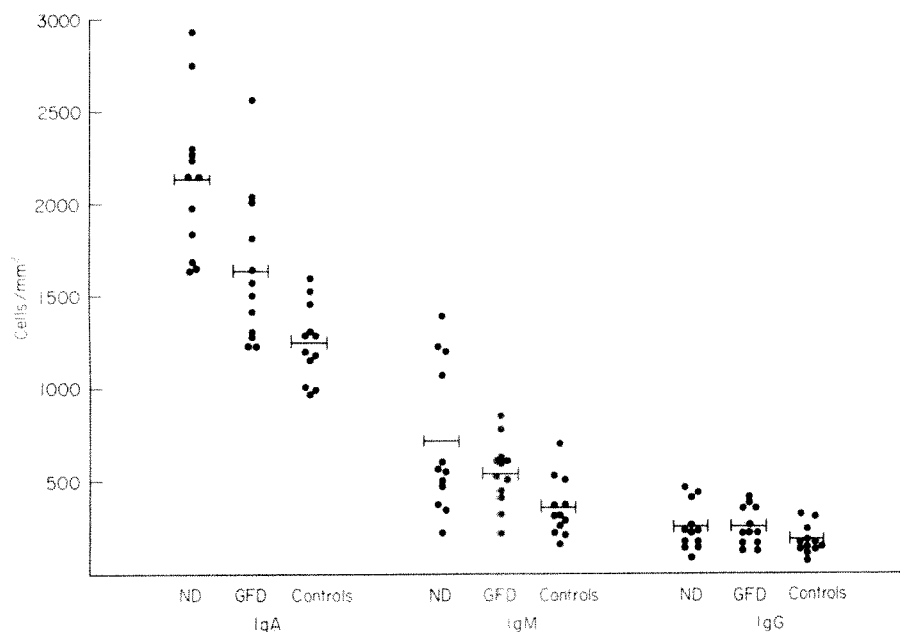


FIGURE 3. Immunoglobulin-containing cells in the jejunal mucosa after treatment in 12 patients on a normal diet (ND) and in 12 patients on a gluten-free diet (GFD).

TABLE 4. Immunoglobulin-containing cells in the jejunal mucosa

Cells	Patients				Controls		Analysis of variance (one-way classification)	
	Normal diet (n=12)	(%)	Gluten-free diet (n=12)	(%)	(n=12)	(%)		
IgA	2129 ± 410*	(70)	1643 ± 400	(68)	1248 ± 208	(71)	18.8	($P < 0.001$)
IgM	708 ± 399	(22)	541 ± 180	(22)	355 ± 156	(19)	5.2	($P < 0.05$)
IgG	250 ± 126	(8)	246 ± 103	(10)	168 ± 65	(10)	2.5	n.s.

* Mean number of cells/mm² ± s.d.

The numbers of Ig-containing cells in the jejunal mucosa are shown in Fig. 3. The cells containing IgA and IgM were most numerous in the normal diet group, i.e. in patients with villous atrophy. The figures were lower in the GFD group but even these exceeded the values in controls (Table 4). The mean numbers of IgG-containing cells were the same in the patient groups but exceeded those in controls. The percent distribution of cells containing IgA, IgM and IgG was about the same in the patient groups and in the controls (Table 4). However, individual variations were large; the proportion of cells containing IgA varied from 54 to 82%, that of cells containing IgM from 11 to 38% and that of cells containing IgG from 3 to 16%. The greatest variations were seen in the normal diet group.

The correlation coefficients between all variables (villous height, intraepithelial lymphocyte count and Ig-containing cells of the jejunal mucosa; serum IgG, IgA, IgM, C₃, C₄ and dapsone; C₃ in the

skin and KI skin test) were calculated for the patient groups. The only significant correlation coefficient was that between C₃ and C₄ ($r=0.88$, $df=10$, $P<0.001$) in the normal diet group.

Strictness of the GFD

At the re-examination 2 patients were found to have failed to follow a strict GFD. Both had ingested at least small amounts of gluten nearly every day. They still needed a large amount of dapsone, 75% and 40% of the dose at the beginning of the diet. They both had C₃ deposits in the skin and signs of an intestinal lesion. One patient was the only one with villous atrophy in the GFD group and both had increased lymphocyte counts. On the other hand, the patient who showed most mucosal improvement in the normal diet group had reduced her gluten intake by excluding bread from her diet. At the same time her daily dapsone requirement decreased from 50 to 25 mg.

DISCUSSION

Recent studies have shown that the rash in DH responds to GFD treatment, which allows a reduction in the dose of the drug required to control skin lesions. As many as 1/3 to 1/2 of the patients treated for a half year or longer with a strict GFD have been found to manage without drugs (Fry *et al.*, 1973; Heading *et al.*, 1976; Reunala *et al.*, 1977). In this study the patients whose skin condition responded to GFD treatment were also found to have a definite improvement of the jejunal lesion. Of the 12 patients only one was found to have villous atrophy after GFD treatment. In contrast, all 12 patients on a normal diet continued to have villous atrophy. An increased intra-epithelial lymphocyte count seemed to be a more sensitive indicator of the mucosal abnormality than did villous architecture. Even after GFD treatment 4 patients had increased lymphocyte counts. This is in accordance with earlier studies of DH and CD (Ferguson & Murray, 1971; Fry *et al.*, 1972).

In the GFD group the immunological findings in the skin, serum and small intestine were fewer in number and/or milder than in patients on a normal diet, obviously indicating a decreased disease activity in patients on a GFD. IgA deposits were found in the uninvolved skin of every patient irrespective of the treatment used, but the fluorescence seemed to be less intense in patients on a GFD. In agreement with this Harrington & Read (1977) recently reported that GFD treatment caused a decrease in the deposition of IgA in all of their 10 patients. Contrary to the present results two of their patients did not have IgA deposits at all after diet treatment. In the present study a clear difference was found in the occurrence of C₃ deposits. Eight patients on a normal diet and 3 on a GFD, two of whom did not follow a strict diet, had C₃ deposits in the uninvolved skin. Prior studies have shown that C₃ deposits are found irrespective of whether involved or uninvolved skin is examined. In a majority of cases C₃ deposits are found in sites corresponding to those of IgA and these deposits are thought to represent immune complexes (van der Meer, 1969; Seah, Mazaheri & Fry, 1973). Moreover, it has been reported recently that sulphones do not have any effect on C₃ deposition (Katz *et al.*, 1976). Thus the results of the present study seem to indicate that the presence of C₃ deposits in the uninvolved skin is related to gluten consumption.

A fall in serum IgM and complement consumption has been observed after gluten challenge in CD (McNeish, Rolles & Thompson, 1974; Lancaster-Smith *et al.*, 1976). Low levels of serum IgM and hypocomplementaemia have also been reported in DH (Fraser *et al.*, 1969; Lancaster-Smith *et al.*, 1974; Mohammed *et al.*, 1976) but no significant alterations in the serum levels of C₃ during the exacerbation or involution of the rash have been observed (Fraser, Beck & Albert-Recht, 1971; Katz *et al.*, 1976). Even though a definite difference was observed in the occurrence of C₃ deposits in the skin of the present patients, the mean levels of C₃ in the serum were within normal limits in both patient groups. The level was, however, lower in the normal diet group though the difference was not signi-

ficant. The mean levels of serum IgG, IgA, IgM and C₄ were within normal limits and similar in both patient groups of this study. The present results showed that patients on a normal diet had more antibodies than patients on a GFD. This agrees with the previous reports concerning the effect of GFD treatment on the presence of ARA and dietary antibodies in DH and CD (Seah *et al.*, 1973; Kumar *et al.*, 1976).

Increased synthesis of IgA, IgM and IgG has been found in organ cultures of jejunal mucosa from patients with DH and CD; at least part of this increase seems to be due to the production of anti-gluten antibodies (Falchuk & Strober, 1974; Gebhard *et al.*, 1974; McClelland *et al.*, 1976). The increase of Ig-containing cells in the jejunal mucosa appears to be a valid expression of the local immunoglobulin synthesis (Lai-A-Fat, Cormane & van Furth, 1974). IF examination of the jejunal mucosa in untreated CD has revealed increased numbers of Ig-containing cells both in children and in adults (Douglas *et al.*, 1970; Savilahti, 1972; Lancaster-Smith *et al.*, 1976; Brandtzaeg & Baklien, 1976). In patients with DH the same seems to be the case. In 11 patients with villous atrophy Lancaster-Smith *et al.* (1974) found a significant increase in cells containing IgM and IgG. In contrast to this, IgA-containing cells were also increased in 6 patients with subtotal villous atrophy studied by Brandtzaeg & Baklien (1976). All three immunocyte classes were also increased in the present patients with villous atrophy, i.e. in patients on a normal diet. However, the increase in IgG-containing cells was not as clear as in patients studied by Brandtzaeg & Baklien, (1976).

GFD treatment causes a reduction in the immunocyte population of the jejunal mucosa in CD (Savilahti, 1972; Lancaster-Smith *et al.*, 1974). In DH a marked decrease in IgM-containing cells was found in two patients treated with a GFD (Lancaster-Smith *et al.*, 1974). In addition, gluten challenge produced an increase in cells containing IgA and IgM in 9 patients with DH (Lancaster-Smith, Kumar & Clark, 1974). In the present study the patients on a GFD had lower numbers of cells containing IgA and IgM than the patients on a normal diet. It can therefore be assumed that also in DH gluten withdrawal diminishes the immunoglobulin synthesis in the jejunum.

In this study the elimination of gluten from the diet caused a significant jejunal improvement which, however, did not lead to complete normality. The mean villous height was lower and the mean numbers of intraepithelial lymphocytes and Ig-containing cells were markedly higher in the GFD group than in the controls. This difference may be due to temporary or continuous ingestion of gluten because it is very difficult even for a co-operative patient to observe a strict GFD (Weinstein *et al.*, 1971; Dissanayake, Truelove & Whitehead, 1974). In fact, even direct questioning on the dietary habits during the GFD often fails to detect a continuous gluten ingestion (Baker, Barry & Read, 1975). In this study two patients on a GFD were known to ingest at least small amounts of gluten and it is possible that some others did the same. If this is true, these small and unrecognized dietary faults might explain why IgA deposits were found in every patient on a GFD although five of them did not need dapsone and did not show any other clinical evidence of active disease.

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REFERENCES

- BAKER, P.G., BARRY, R.E. & READ, A.E. (1975) Detection of continuing gluten ingestion in treated coeliac patients. *British Medical Journal*, **i**, 486.

- BEUTNER, E.H. & NISENGARD, R.J. (1973) Defined immunofluorescence in clinical immunopathology. In: *Immunopathology of the Skin. Labelled Antibody Studies*, p.197. Dowden, Hutchinson & Ross, Stroudsburg, U.S.A.
- BRANDTZAEG, P. & BAKLIEN, K. (1976) Immunoglobulin-producing cells in the intestine in health and disease. In: *Clinics in Gastroenterology*, Vol. 5, p.251. W.B.Saunders, London.
- DISSANAYAKE, A.S., TRUELOVE, S.C. & WHITEHEAD, R. (1974) Jejunal mucosal recovery in coeliac disease in relation to the degree of adherence to a gluten-free diet. *Quarterly Journal of Medicine*, **43**, 161.
- DOUGLAS, A.P., CRABBE, P.A. & HOBBS, J.R. (1970) Immunochemical studies of the serum, intestinal secretions and intestinal mucosa in patients with adult coeliac disease and other forms of the coeliac syndrome. *Gastroenterology*, **59**, 414.
- FALCHUK, Z.M. & STROBER, W. (1974) Gluten-sensitive enteropathy: synthesis of antigliadin antibody *in vitro*. *Gut*, **15**, 947.
- FERGUSON, A. & MURRAY, D. (1971) Quantitation of intraepithelial lymphocytes in human jejunum. *Gut*, **12**, 988.
- FRASER, N.G., DICK, H.M. & CRICHTON, W.B. (1969) Immunoglobulins in dermatitis herpetiformis and various other skin diseases. *British Journal of Dermatology*, **81**, 89.
- FRASER, N.G., BECK, J.S. & ALBERT-RECHT, F. (1971) Serum complement (C' 3) and immunoglobulin levels in dermatitis herpetiformis. *British Journal of Dermatology*, **85**, 314.
- FRY, L., MCMINN, R.M.H., COWAN, J.D. & HOFFBRAND, A.V. (1968) Effect of gluten-free diet on dermatological, intestinal, and haematological manifestations of dermatitis herpetiformis. *Lancet*, **i**, 557.
- FRY, L., SEAH, P.P., MCMINN, R.M.H. & HOFFBRAND, A.V. (1972) Lymphocytic infiltration of epithelium in diagnosis of gluten-sensitive enteropathy. *British Medical Journal*, **iii**, 371.
- FRY, L., SEAH, P.P., RICHES, D.J. & HOFFBRAND, A.V. (1973) Clearance of skin lesions in dermatitis herpetiformis after gluten withdrawal. *Lancet*, **i**, 288.
- GEBHARD, R.L., FALCHUK, Z.M., KATZ, S.I., SESSOMS, C., ROGENTINE, G.N. & STROBER, W. (1974) Dermatitis herpetiformis. Immunologic concomitants of small intestinal disease and relationship to histocompatibility antigen HL-A8. *Journal of Clinical Investigation*, **54**, 98.
- HARRINGTON, C.I. & READ, N.W. (1977) Dermatitis herpetiformis: effect of gluten-free diet on skin IgA and jejunal structure and function. *British Medical Journal*, **i**, 872.
- HEADING, R.C., PATERSON, W.D., MCCLELLAND, D.B.L., BARNETSON, R.St.C. & MURRAY, M.S.M. (1976) Clinical response of dermatitis herpetiformis skin lesions to a gluten-free diet. *British Journal of Dermatology*, **94**, 509.
- KATZ, S.I., HERTZ, K.C., CRAWFORD, P.S., GAZZE, L.A., FRANK, M.M. & LAWLEY, T.J. (1976) Effect of sulfones on complement deposition in dermatitis herpetiformis and on complement-mediated guinea-pig reactions. *Journal of Investigative Dermatology*, **67**, 688.
- KUMAR, P.J., FERGUSON, A., LANCASTER-SMITH, M. & CLARK, M.L. (1976) Food antibodies in patients with dermatitis herpetiformis and adult coeliac disease—relationship to jejunal morphology. *Scandinavian Journal of Gastroenterology*, **11**, 5.
- LAI-A-FAT, R.F.M., CORMANE, R.H. & VAN FURTH, R. (1974) An immunohistopathological study on the synthesis of immunoglobulins and complement in normal and pathological skin and adjacent mucous membranes. *British Journal of Dermatology*, **90**, 123.
- LANCASTER-SMITH, M., KUMAR, J. & CLARK, M.L. (1974) Immunological phenomena following gluten challenge in the jejunum of patients with adult coeliac disease and dermatitis herpetiformis. In: *Coeliac Disease. Proceedings of the 2nd International Coeliac Symposium*, p.173. Stenfert Kroese, Leiden.
- LANCASTER-SMITH, M., KUMAR, P., MARKS, R., CLARK, M.L. & DAWSON, A.M. (1974) Jejunal mucosal immunoglobulin-containing cells and jejunal fluid immunoglobulins in adult coeliac disease and dermatitis herpetiformis. *Gut*, **15**, 371.
- LANCASTER-SMITH, M., PACKER, S., KUMAR, P.J. & HARRIES, J.T. (1976) Immunological phenomena in the jejunum and serum after reintroduction of dietary gluten in children with treated coeliac disease. *Journal of Clinical Pathology*, **29**, 592.
- MARKS, J., SHUSTER, S. & WATSON, A.J. (1966) Small-bowel changes in dermatitis herpetiformis. *Lancet*, **ii**, 1280.
- MEER, J.B. VAN DER (1969) Granular deposits of immunoglobulins in the skin of patients with dermatitis herpetiformis. An immunofluorescent study. *British Journal of Dermatology*, **81**, 493.
- MCCLELLAND, D.B.L., BARNETSON, R.St.C., PARKIN, D.M., WARWICK, R.R.G., HEADING, R.C. & SHEARMAN, D.J.C. (1972) Small-intestinal immunoglobulin levels in dermatitis herpetiformis. *Lancet*, **ii**, 1108.
- MCCLELLAND, D.B.L., SHEARMAN, D.J.C., LAI-A-FAT, R.F.M. & VAN FURTH, R. (1976) In vitro synthesis of immunoglobulins, secretory component and lysozyme by human gastrointestinal tissues. II. Pathological tissues. *Clinical and Experimental Immunology*, **23**, 20.
- MCNEISH, A.S., ROLLES, C.J. & THOMPSON, R.A. (1974) Complement and its degradation products after challenge

- with gluten in children with coeliac disease. In: *Coeliac Disease. Proceedings of the 2nd International Coeliac Symposium*, p.197. Stenfert Kroese, Leiden.
- MOHAMMED, I., HOLBOROW, E.J., FRY, L., THOMPSON, B.R., HOFFBRAND, A.V. & STEWART, J.S. (1976) Multiple immune complexes and hypocomplementaemia in dermatitis herpetiformis and coeliac disease. *Lancet*, **ii**, 487.
- REUNALA, T., SALO, O.P., TIILIKAINEN, A. & MATTILA, M.J. (1976a) Histocompatibility antigens and dermatitis herpetiformis with special reference to jejunal abnormalities and acetylator phenotype. *British Journal of Dermatology*, **94**, 139.
- REUNALA, T., SALO, O.P., TIILIKAINEN, A., SELROOS, O., & KUITUNEN, P. (1976b) Family studies in dermatitis herpetiformis. *Annals of Clinical Research*, **8**, 254.
- REUNALA, T., BLOMQUIST, K., TARPILA, S., HALME, H. & KANGAS, K. (1977) Gluten-free diet in dermatitis herpetiformis. I. Clinical response of skin lesions in 81 patients. *British Journal of Dermatology*, **97**, 473.
- SAVILAHTI, E. (1972) Intestinal immunoglobulins in children with coeliac disease. *Gut*, **13**, 958.
- SEAH, P.P., FRY, L., STEWART, J.S., CHAPMAN, B.L., HOFFBRAND, A.V. & HOLBOROW, E.J. (1972) Immunoglobulins in the skin in dermatitis herpetiformis and coeliac disease. *Lancet*, **i**, 611.
- SEAH, P.P., FRY, L., HOLBOROW, E.J., ROSSITER, M.A., DOE, W.F., MAGALHAES, A.F. & HOFFBRAND, A.V. (1973) Antireticulin antibody: incidence and diagnostic significance. *Gut*, **14**, 311.
- SEAH, P.P., MAZAHARI, M.R. & FRY, L. (1973) Complement in the skin of patients with dermatitis herpetiformis. *British Journal of Dermatology*, **89**, suppl. 9, 12.
- SEAH, P.P. & FRY, L. (1975) Immunoglobulins in the skin in dermatitis herpetiformis and their relevance in diagnosis. *British Journal of Dermatology*, **92**, 157.
- WEINSTEIN, W.M., BROW, J.R., PARKER, F. & RUBIN, C.E. (1971) The small intestinal mucosa in dermatitis herpetiformis. II. Relationship of the small intestinal lesion to gluten. *Gastroenterology*, **60**, 362.

Rheumatoid factor in sera of dermatitis herpetiformis patients

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SUMMARY

Serum specimens from 44 patients with dermatitis herpetiformis (DH) and 42 control patients were analyzed with solid-phase radioimmunoassay (RIA) developed to detect IgG and IgM class antibodies against herpes simplex virus (HSV) envelope antigen. They were also tested with different tests to detect rheumatoid factor (RF) as well as serum antibodies to gastric parietal cells, glomerular basement membrane, smooth muscle, mitochondria and nuclei. Total serum IgG, IgM and IgA quantification was performed concurrently.

IgM class antibodies reacting in HSV envelope antigen RIA were found in 47.8% of the DH patients, but in none of the controls. The age distribution of the IgM positive patients was in accordance with the appearance of RF, representing elderly persons. The presence of RF in serum specimens was confirmed by removing the IgM reactivity with heat aggregated human gamma globulin adsorption and in 13 patients with positive serological tests for RF. No correlation was found with the appearance of RF and duration or severity of the disease or with the detection of RF and serum total IgM concentration. In DH patients antibodies to gastric parietal cells were found in 6 and to smooth muscle in 1 of 32 specimens tested.

Several facts indicate a possible autoimmune aetiopathology in dermatitis herpetiformis (DH). Deposits of IgA class immunoglobulins in the dermal papillae of uninvolved skin are considered pathognomonic for the disease (Van der Meer, 1969; Chorzelsky *et al.*, 1971; Holubar, Doralt & Eggerth, 1971; Seah *et al.*, 1972; Fry & Seah, 1974; Seah & Fry, 1975). The real significance of these immunoglobulins in the pathogenesis of DH is, however, still obscure. Seah *et al.* (1971, 1973) and Lancaster-Smith *et al.* (1975) detected circulating antibodies to reticulin in DH patients. Serum antinuclear antibodies and antibodies against thyroid and gastric parietal cells have also been found (Fraser, 1970; Seah *et al.*, 1971). Mowbray *et al.* (1973) and later Mohammed *et al.* (1976) also demonstrated circulating immune complexes in DH patients.

Recently a solid-phase radioimmunoassay (RIA) was developed in our laboratory to detect IgG and IgM class antibodies against herpes simplex virus (HSV) (Kalimo *et al.*, 1977a). The technique

proved sensitive and specific in acute infections; a rapid development and decline of IgM antibodies and a slower development of IgG antibodies were apparent. The same methodology was later adapted to detect class specific antibodies against HSV capsid, envelope and excreted antigens (Kalimo *et al.*, 1977b). In the assays with HSV envelope antigen, DH patients seemed to form a peculiar group possessing IgM class antibodies reacting to the test. This finding prompted further studies of these patients with the HSV envelope antigen RIA and concurrently with the other serological methods available to prove the specificity of the IgM reactive antibodies. The results of these studies are described below.

PATIENTS AND METHODS

Patients. The serum specimens were obtained from the Department of Dermatology, University of Turku, comprising the following: (1) 58 specimens from 44 patients with DH (male 30, female 14, age range 19–70 years); two or three specimens were obtained from seven patients with a minimum interval of 2 months. (2) Two specimens from 2 patients with suspected DH (male and female, age 65 and 70 years). (3) Thirty specimens from 30 control patients with no history of skin problems, but with a previous exposure to HSV infection (male 14, female 16, age range 19–70 years). (4) Five specimens from 5 females with lichen simplex chronicus (age range 40–61 years). (5) Five specimens from 5 patients with atopic dermatitis (male 2, female 3, age range 21–74 years).

From the 58 DH specimens 13 were obtained before any medication or diet was started; the rest of the specimens were obtained whilst the patients were advised to follow a gluten free diet and/or were taking dapsone.

Two of the DH patients had an additional diagnosis of rheumatoid arthritis and two had discoid lupus erythematosus. The serum specimens were stored at -20°C until used.

Serological tests. The quantitative determination of serum IgG, IgM and IgA immunoglobulins was performed by the radial immunodiffusion technique (Mancini, Carbonara & Heremans, 1965). Antisera were obtained from Behringwerke Ab (Mahrburg-Lahn). Standardizations were made according to WHO standard preparation 67/97.

To demonstrate rheumatoid factor (RF), human group O Rh positive cells were coated with rabbit antihuman erythrocyte serum (Waler-Rose), with baboon antihuman fibroblast serum (Baboon) or with human anti-D (Ripley). The Latex test was carried out with Hyland's RA test latex-globulin reagent. For all titrations U-microtiter equipment was used. Additional details are described elsewhere (Pyrhönen *et al.*, 1976). Antibodies to nuclei, smooth muscle, gastric parietal cells, glomerular basement membrane and mitochondria were measured using standard indirect immunofluorescent methods. Unfixed tissue sections of rat stomach, kidney (Andersen & Beutner, 1972; Andersen, 1974) and liver (Holborow, Weir & Johnson, 1957) were used to demonstrate the tissue antibodies.

Heat aggregated human gamma globulin was used to remove the RF as previously described (Shirodaria *et al.*, 1973).

The RIA for determining the IgG and IgM class antibodies against HSV envelope antigen has been presented in detail earlier (Kalimo *et al.*, 1977b). In brief, the viral antigen was adsorbed onto polystyrene balls, which were then exposed to serial dilutions of test serum. The presence of the antibody was measured by its capacity to bind ^{125}I -labelled specific anti-human IgG or IgM. The titres are expressed as reciprocals and as $\log_2$ values of the reciprocals.

The mean titre values were compared with the Student's *t*-test. The age distribution of the patients and the appearance of IgM class antibodies were compared with the χ^2 -test.

RESULTS

The results of quantification of serum total IgG, IgM and IgA concentration from 58 specimens of 44 DH patients can be summarized as follows: increased IgG levels were demonstrated in three patients; one had a deficient level. IgM level was below the normal limit in 8 patients, and above in one patient. Increased IgA titres were found in 12 patients; none showed a decreased level.

IgG class antibodies against HSV envelope antigen were detected in 87.0% of the DH patients. The mean antibody titre was 11,585 (13.5 ± 1.3 , $\log_2$). Each of the 30 control patients with a previous history of HSV infection had these antibodies with a mean titre of 14 263 (13.8 ± 1.3 , $\log_2$). All five patients with lichen simplex chronicus demonstrated IgG class antibodies to HSV; the mean titre was 15 286 (13.9 ± 1.7 , $\log_2$). Three patients with atopic dermatitis were negative in HSV RIA; the mean titre of the other two was 16 838 (14.0 ± 1.0 , $\log_2$). There were no statistically significant differences in the antibody levels between the different patient groups. No correlation between the titre of IgG class antiviral antibodies and the total serum IgG concentration was found.

IgM class antibodies reacting in HSV envelope antigen RIA were detected in 23 specimens from 20 patients with confirmed DH and in two patients with suspected DH. The mean titre of IgM antibodies was 445 (8.8 ± 2.7 , $\log_2$). Each of these specimens also showed IgG class antibodies. None of the control specimens possessed IgM class antibodies. The total serum IgM concentration was within normal limits in the specimens, demonstrating IgM class antibodies in HSV RIA. The serum specimens were obtained from 13 patients before diet and/or medication was started. IgM class antibodies were found in six patients; one specimen was negative in both HSV IgG and IgM RIA. No significant correlations with the severity of the disease, duration, and appearance of IgM class antibodies could be found. A statistically significant difference ($P < 0.001$) was found in the age distribution of the patients with and without IgM antibodies. Of the patients demonstrating IgM antibodies, 84% were over 40 years as compared with the IgM negative group, in which 55.2% exceeded the age of 40 years. The four patients with rheumatoid arthritis and discoid lupus erythematosus were each positive for IgM class antibodies.

TABLE 1. Results of the Waaler-Rose (WaRo), Latex, Ripley, and Baboon tests and of antibody detection against gastric parietal cells and smooth muscle in dermatitis herpetiformis patients with and without IgM class antibodies reacting in herpes simplex virus envelope antigen radioimmunoassay. The number of positive specimens is indicated in each category

	No. of specimens tested	WaRo	Latex	Ripley	Baboon	Antibodies to	
						Parietal cells	Smooth muscle
HSV-IgM positive	25	4	12	16	11	5	1
HSV-IgM negative	7	0	0	1	0	1	0

To examine the specificity of HSV IgM RIA reactive antibodies and to find out the presence of RF all 25 positive and seven negative specimens were subjected to further studies. The results of these tests are demonstrated in Table 1. In the same table are included the positive results of other autoimmune antibodies detected in these specimens. Four out of the 25 IgM positive specimens tested were positive in the Waaler-Rose test, 12 in the Latex test, 16 in the Ripley and 11 in the Baboon tests. In 13 specimens two tests were found to be positive simultaneously indicating the presence of RF. Parietal cell antibodies were found in 5 patients and smooth muscle antibodies in 1 patient.

Of the HSV-IgM negative patients, one demonstrated parietal cell antibodies and one was positive in Ripley. Antibodies against glomerular basement membrane, mitochondria or nuclei were not found in these specimens. The IgM reactivity could also be removed by adsorbing the serum specimens with heat aggregated human gamma globulin, confirming the presence of RF.

DISCUSSION

Circulating immune complexes have been found in up to 100% of DH patients (Mohammed *et al.* 1976). The majority of the complexes contain both IgG and IgM (46%), 22% contain only IgM and 1% only IgG immunoglobulins. On the basis of these findings it appears evident that in the sera of DH patients IgM class anti-immunoglobulins, RFs should be detected. According to earlier findings RF, are known to cause false positive results in immunofluorescent tests for viral IgM antibodies (Fraser *et al.*, 1971; Shirodaria *et al.*, 1973; Nikoskelainen & Hänninen, 1973). They also interact with infectious HSV-antibody complexes (Ashe *et al.*, 1971).

The RIA method used in this study is in principle analogous to the immunofluorescence test, and thus false positive IgM results caused by RF were suspected. The results of the present study clearly demonstrate that in patients with DH IgM class antibodies could be detected in 47.8% of the patients. None of the controls with a previous history of HSV infection showed IgM class antibodies, nor did patients with lichen simplex chronicus or atopic dermatitis possess IgM antibodies detectable by this technique. The specificity of the IgM antibodies was further tested by the Latex, Waaler-Rose, Baboon or Ripley tests. The presence of RF was confirmed in 13 specimens, in which two tests were found to be positive simultaneously. Furthermore IgM reactivity could be removed from each specimen by heat aggregated human gammaglobulin treatment confirming the presence of RF. The greater sensitivity of the RIA test explains the difference in detecting RF with the different tests used. However, no correlation was found with the severity of the disease, duration or appearance of IgM class antibodies. On the other hand, patients possessing IgM class antibodies, RFs were significantly older than patients who were sero-negative. Of the other autoantibodies tested, gastric parietal cell antibodies were found in six and smooth muscle antibodies in one specimen out of the 32 tested.

Though this study was initially prompted by the results obtained in HSV-IgM envelope antigen RIA, no evidence was obtained of the possible association of DH and HSV. In this case the HSV IgM RIA reactive antibodies in the serum of DH patients were IgM class anti-immunoglobulins, RFs which were detected with the sensitive test.

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This study was supported by the Essex corporation. I am grateful to Dr Kari Penttinen for the analysis of serum specimens by the Latex, Waaler-Rose, Ripley and Baboon tests and for detection of autoantibodies and to Dr Kerttu Irjala for the quantification of serum immunoglobulins. The skilful technical assistance of Ms Marita Maaronen is gratefully acknowledged.

REFERENCES

- ANDERSEN, P. (1974) Indirect immunofluorescence studies of smooth muscle antibodies. *Acta pathologica microbiologica Scandinavica, Section B*, **82**, 577.
- ANDERSON, P. & BEUTNER, E.H. (1972) Defined immunofluorescent studies of cytoplasmic antibodies. *International Archives of Allergy*, **43**, 780.
- ASHE, W.K., DANIELS, C.A., SCOTT, G.S. & NOTKINS, A.L. (1971) Interaction of rheumatoid factor with infectious herpes simplex virus-antibody complexes. *Science*, **172**, 176.

- CHORZELSKI, T.P., BEUTNER, E.H., JABLONSKA, S., BLASZCZYK, M. & TRIFTSHAUSER, C. (1971) Immunofluorescence studies in the diagnosis of dermatitis herpetiformis and its differentiation from bullous pemphigoid. *Journal of Investigative Dermatology*, **56**, 373.
- FRASER, N.G. (1970) Autoantibodies in dermatitis herpetiformis. *British Journal of Dermatology*, **83**, 46.
- FRASER, K.B., SHIRODARIA, P.V. & STANFORD, C.F. (1971) Fluorescent staining and human IgM. *British Medical Journal*, **iii**, 707.
- FRY, L. & SEAH, P.P. (1974) Dermatitis herpetiformis: an evaluation of diagnostic criteria. *British Journal of Dermatology*, **90**, 137.
- HOLBOROW, E.J., WEIR, D.M. & JOHNSON, G.D. (1957) A serum factor in lupus erythematosus with affinity for tissue nuclei. *British Medical Journal*, **ii**, 732.
- HOLUBAR, K., DORALT, M. & EGGERTH, G. (1971) Immunofluorescence patterns in dermatitis herpetiformis. *British Journal of Dermatology*, **85**, 505.
- KALIMO, K.O.K., ZIOLA, B., VILJANEN, M., GRANFORS, K. & TOIVANEN, P. (1977a) Solid-phase radioimmunoassay of herpes simplex virus IgG and IgM antibodies. *Journal of Immunological Methods*, **14**, 83.
- KALIMO, K.O.K., MARTTILA, R., GRANFORS, K. & VILJANEN, M. (1977b) Solid-phase radioimmunoassay of human Immunoglobulin M and Immunoglobulin G antibodies against herpes simplex virus type 1 capsid, envelope and excreted antigens. *Infection and Immunity*, **15**, 883.
- LANCASTER-SMITH, M., KUMAR, P., CLARK, M.L., MARKS, R. & JOHNSON, G.D. (1975) Antireticulin antibodies in dermatitis herpetiformis and adult coeliac disease. *British Journal of Dermatology*, **92**, 37.
- MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.
- MOHAMMED, I., HOLBOROW, E.J., FRY, L., THOMPSON, B.R., HOFFBRAND, A.V. & STEWART, J.S. (1976) Multiple immune complexes and hypocomplementaemia in dermatitis herpetiformis and coeliac disease. *Lancet*, **ii**, 487.
- MOWBRAY, J.F., HOFFBRAND, A.V., HOLBOROW, E.J. & SEAH, P.P. (1973) Circulating immune complexes in dermatitis herpetiformis. *Lancet*, **i**, 400.
- NIKOSKELAINEN, J. & HÄNNINEN, P. (1975) Antibody response to Epstein-Barr virus infectious mononucleosis. *Infection and Immunity*, **11**, 42.
- PYRHÖNEN, S., TIMONEN, T., HEIKKINEN, A., PENTTINEN, K., ALFTAN, O., SAKSELA, E. & WAGER, O. (1976) Rheumatoid factor as an indicator of serum blocking activity and tumour recurrences in bladder tumours. *European Journal of Cancer*, **12**, 87.
- SEAH, P.P., FRY, L., HOFFBRAND, A.V. & HOLBOROW, E.J. (1971) Tissue antibodies in dermatitis herpetiformis and adult coeliac disease. *Lancet*, **i**, 834.
- SEAH, P.P., FRY, L., STEWART, J.S., CHAPMAN, B.L., HOFFBRAND, A.V. & HOLBOROW, E.J. (1972) Immunoglobulins in the skin in dermatitis herpetiformis and coeliac disease. *Lancet*, **i**, 611.
- SEAH, P.P., FRY, L., HOLBOROW, E.J., ROSSITER, M.A., DOE, W.F., MAGALHAES, A.F. & HOFFBRAND, A.V. (1973) Antireticulin antibody: incidence and diagnostic significance. *Gut*, **14**, 311.
- SEAH, P.P. & FRY, L. (1975) Immunoglobulins in the skin in dermatitis herpetiformis and their relevance in diagnosis. *British Journal of Dermatology*, **92**, 157.
- SHIRODARIA, P.V., FRASER, K.B. & STANFORD, F. (1973) Secondary fluorescent staining of virus antigens by rheumatoid factor and fluorescein-conjugated anti-IgM. *Annals of the Rheumatic Diseases*, **32**, 53.
- VAN DER MEER, J.B. (1969) Granular deposit of immunoglobulins in the skin of patients with dermatitis herpetiformis. An immunofluorescent study. *British Journal of Dermatology*, **81**, 493.

Psoriasis in Sri-Lanka—a computer analysis of 1366 cases

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SUMMARY

A clinical and epidemiological study of psoriasis in Sri-Lanka is presented. The prevalence in the population is estimated to be over 0.4%. The clinic incidence was 8.7%; of these 53.2% were male, and 46.8% female and 8.3% reported a family history of the disease. The mean age at onset was 25.2 years. The frequency distribution for the age at onset was bimodal with peaks at the second and fifth decades. The age at onset was significantly low in females, in patients with a family history of the disease, in those affected by weather changes and in patients whose disease was precipitated by sore throats. The existence of natural subpopulations of patients is suggested. Clinical types and complications of the disease were not essentially different from those observed in the West.

Clinical and epidemiological data on psoriasis are available from Northern European countries (Ingram, 1954; Lomholt, 1963; Hellgren, 1964), the U.S.A. (Farber & Nall, 1974), and from other geographical locations: Kenya (Verhagen & Koten, 1967), Uganda (Lomholt, 1971b), Tanzania (Masawe, 1973), Brazil (Azulay, 1976), South America (Kerdal-Vegas, 1971), Japan (Yasuda, Ischikawa & Mori, 1971) and East Pakistan (Khaleque & Basit, 1968). No such data are available from Sri-Lanka. A study was therefore done to determine the clinical and epidemiological characteristics of psoriasis in Sri-Lanka. This study is based on a computer analysis of 1366 consecutive case records of patients with psoriasis. These patients were seen personally by one of us over a period of 6 years and 4 months at a hospital dermatological clinic in Kandy, Sri-Lanka.

THE COUNTRY AND THE PEOPLE

Sri-Lanka, known as Ceylon until 1972, is an island state in the Indian Ocean situated just off the extreme south-east coast of the Indian subcontinent, between 6° and 10° North latitude. It is 25,000 square miles in extent. Although the island lies close to the equator, its position in relation to the Indian subcontinent, the surrounding seas and also its own relief gives it a modified equatorial climate.

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The population of the island is made up of various ethnic groups (Table 1). The Sinhalese, speaking a language of Indo-Aryan origin, form the major element. The Tamils, speaking a Dravidian language, are mainly of South Indian origin. The Moors are descendants of Indian and Arab traders and seafarers. The others are mostly Burghers who are descendants of various European races, mainly of the Dutch (Ludowyke, 1962).

TABLE 1. The ethnic distribution of the general population of Sri-Lanka, the Health Division of Kandy (*Statistical Abstracts of Ceylon*, 1969), 3000 consecutive patients attending the skin clinic and the patients with psoriasis

	Sinhalese	Tamils	Moors	Others
Sri-Lanka	9,146,679 (71.9%)	2,611,935 (20.5%)	851,707 (6.7%)	59,822 (0.7%)
Kandy	924,739 (56.4%)	596,757 (36.4%)	109,931 (6.7%)	8,986 (0.5%)
Skin clinic	2,529 (84.3%)	213 (7.1%)	240 (8.0%)	18 (0.6%)
Psoriasis	1,039 (76.0%)	184 (13.5%)	124 (9.1%)	19 (1.4%)

MATERIALS AND METHODS

Kandy is the second largest city in the island and is situated in the central highlands at an altitude of 450 m. The Kandy General Hospital is the largest medical institution in the Health Division of Kandy. The hospital has 1126 beds and an annual turnover of 71,000 in-patients and 547,000 out-patients. The dermatological unit of this hospital is the only one in the Health Division of Kandy, and serves a population of nearly 1.7 million.

The patients seen at this clinic were mostly referred from the general out-patients department where between 1000 and 1500 patients are seen daily. Patients referred from other specialized clinics, from neighbouring district and provincial hospitals, and by the general practitioners in the area were also seen. All patients apart from acute cases were seen by appointment. Most patients seen suffered from severe dermatoses. Clinics for venereal diseases and leprosy were run separately. All patients seen at the skin clinic were interviewed and examined by D.A.G.

By December 1972 (i.e. after a period of 6 years and 4 months) 16,873 case records of patients were available for analysis. Among them 1465 case records carried a diagnosis of psoriasis. In most instances the diagnosis was made clinically.

The 1465 case records were reviewed and 57 items of information gleaned from the records were coded alpha-numerically and placed on punch cards. The data were processed using an IBM 1130 computer. The cards were first subjected to a checking programme to detect logical errors in coding and punching and the data were stored on magnetic disc. The records were then sorted according to age, sex, ethnic group and name so as to detect any duplicated records. (By duplication is meant that more than one case record was found for a single patient.) Data from the duplicated records were merged and the disc file updated. The total number of records then available for analysis was 1366.

RESULTS

Frequency of occurrence

Table 2 shows the relative incidence of the five most common skin diseases at the skin clinic, Kandy. Assuming that the duplication rates for the other diseases were the same as that for psoriasis the clinic incidence was 8.7%.

TABLE 2. Relative incidence of the five most common skin diseases. (Dermatological Clinic, General Hospital, Kandy, Sri-Lanka, Aug. 1966 to Dec. 1972)

Disease	Number	Percent
Infective eczematoid dermatitis	2307	13.7
Scabies	1917	11.4
Psoriasis	1465	8.7
Contact dermatitis	1341	8.1
Vitiligo	764	4.5

The 1366 patients were drawn from a population of 1.7 million. Thus the minimum prevalence of psoriasis in the Health Division of Kandy was 0.08%. However, since distance may have precluded many patients in the Health Division attending the clinic, the prevalence was determined for the population of the Kandy municipality which is within 5 miles radius of the skin clinic. The mean mid-year population of the municipality in 1972 was 93,000 and 412 (30%) patients were from this population. This gives a minimum prevalence rate of 0.4% for those living within this municipality.

Distribution by sex

727 (53.2%) were male while 693 (46.8%) were female. This male preponderance was not statistically significant when compared with the general population.

Ethnic distribution

Table 1 shows the distribution of patients by ethnic groups. When the ethnic distribution of the clinic patients was compared with the ethnic distribution of patients with psoriasis, it was found that there were proportionately less Sinhalese and more Tamils among psoriatics ($P < 0.001$). This difference may be due to there being less Tamils among patients with other skin diseases or more Tamils among patients with psoriasis or both. The proportion of Moors showed no significant difference.

Age at first visit

The mean age at first visit for females was 25.0 years and for males 34.7 years. The youngest patient was a 1.5-year-old female with psoriasis of the scalp and the oldest was an 88-year-old male with plantar psoriasis. The median age at first visit was 26 years. When the frequency distribution for the age at first visit was plotted, it was noted that prominent peaks occurred at multiples of 5 years. These peaks were more obvious in the older age groups, perhaps due to the patients rounding off their ages to the nearest multiple of five. To smooth out these fluctuations of the frequency distribution 5-year moving averages were used. Fig. 1 shows the frequency distribution of the age at first visit plotted as a 5-year moving average and also as a histogram by 5-year age blocks. The curve is bimodal with peaks at 18 and 40 years. This bimodality is less clear on the histogram.

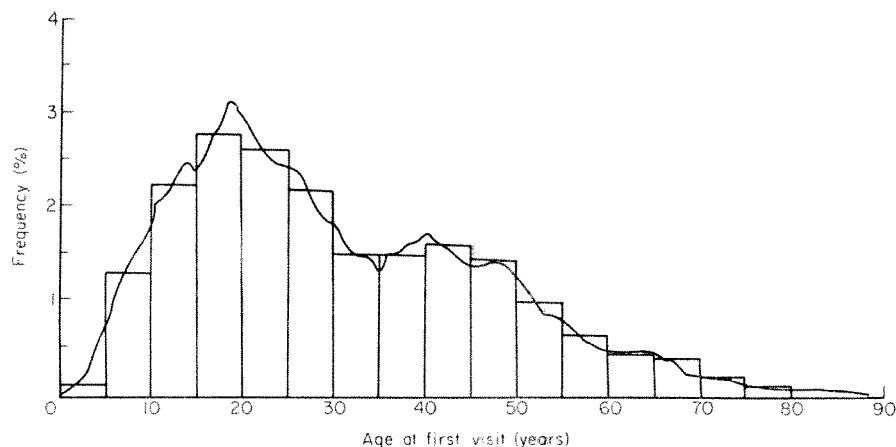


FIGURE 1. Frequency distribution of the age at first visit plotted as a 5-year moving average curve and also as a histogram by 5-year age blocks. Frequency is expressed as a percentage. The histogram is scaled down by a fifth to enable comparison with the curve.

Age at onset

This figure was derived by subtracting the duration of the disease at the time of first visit from the age at first visit. 80 patients (5.9%) could not recall the duration of the disease. The age at onset could thus be determined in only 1286 (94.1%) patients. In 3 patients the history of psoriasis dated back to infancy and one patient developed the disease at 88 years. The mean age at onset was 25.2 years (s.d. = 15.4, median = 21.4).

Fig. 2 shows the frequency distribution of the age at onset for 1286 patients. There is a steeply rising incidence of onset after 3 years of age reaching a peak at 19 years. Another peak is observed at 41 years. The frequency distribution of the age of the general population is given in the same figure for comparison. The two peaks in the age of psoriasis onset could be true peaks rather than manifestations of the age structure of the population.

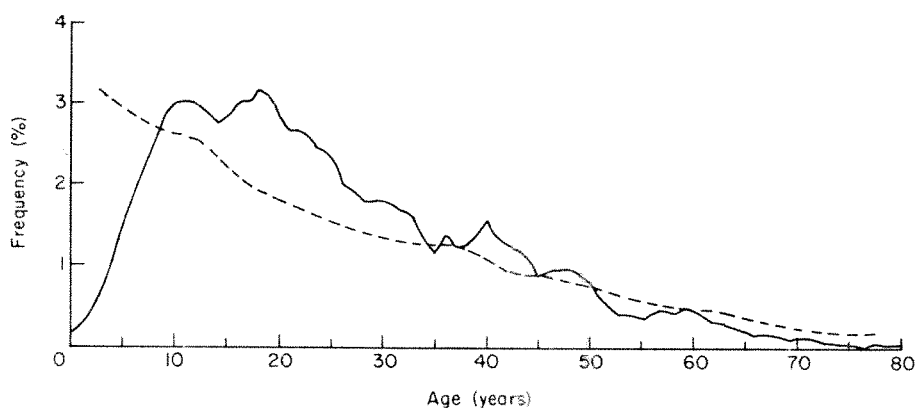


FIGURE 2. Frequency distribution of the age at onset of psoriasis compared with the frequency distribution of the general population of Sri-Lanka (*Statistical Abstracts of Ceylon*, 1969). Both curves are plotted as 5-year moving averages and in both curves frequency is expressed as a percentage. (—) Psoriasis onset; (---) general population.

Age at onset for males and females

The age at onset could be determined in 681 of the 727 male patients. Their mean age at onset was 29.6 years (s.d. = 16.1, median = 27.0, range = 0-88). The age at onset was determined for 605 of the 639 female patients. The mean age at onset for females was 20.4 years (s.d. = 12.9, median = 16.5, range = 1.5-66). The difference of 9.2 years between the mean ages of onset for males and females was statistically significant ($P < 0.001$). Fig. 3 shows the frequency distributions of the age at onset for males and females separately.

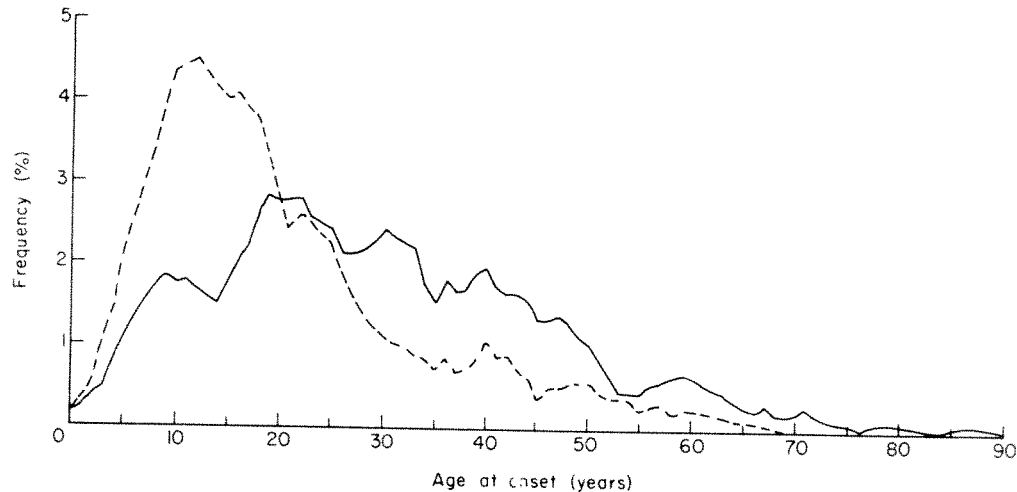


FIGURE 3. Frequency distribution of the age at onset for males and females plotted as 5-year moving averages. (—) Male; (---) female.

Age at onset and ethnic groups

The mean age at onset for Sinhalese was 28.9 years (s.e.m. = 0.50), for Tamils 24.8 years (s.e.m. = 1.23) and for Moors 28.9 years (s.e.m. = 1.49). The difference of 4.1 years between the Tamils and the other two ethnic groups was statistically significant ($P = 0.002$ and 0.034 respectively).

Familial occurrence

Patients were interviewed regarding the occurrence of psoriasis in parents, siblings, children, grandparents, first cousins, aunts, uncles and the numbers so affected. 970 (71.0%) stated that none of their family members were affected. 282 (20.6%) were uncertain of any familial occurrence. 114 (8.3%) had one or more members of their family affected.

Family history and age at onset

The age at onset could be determined in 112 out of 114 patients with a family history and in 961 of the 970 patients without a family history. The mean age at onset for those with a family history was 17.2 years (s.d. = 9.4, median = 15.3, range = 3-50), and for patients without a family history was 25.3 years (s.d. = 15.0, median = 21.8, range = 0-88). This difference of 8.1 years was statistically significant ($P < 0.001$). Fig. 4 shows the frequency distribution curves for the patients with and without a family history. Table 3 shows the mean ages at onset for both sexes, for those with and without a family history and the statistical significance of these differences. Further, the Kruskal-Wallis analysis of variance of ranks and the median test showed that the differences in the relative frequencies in the respective subgroups are due to some kind of systematic influence rather than due to

chance fluctuation. Fig. 5 shows the frequency distribution curves for the age at onset for males and females with and without a family history of psoriasis. Almost all the females with a family history of psoriasis are concentrated within a narrow peak in the second decade.

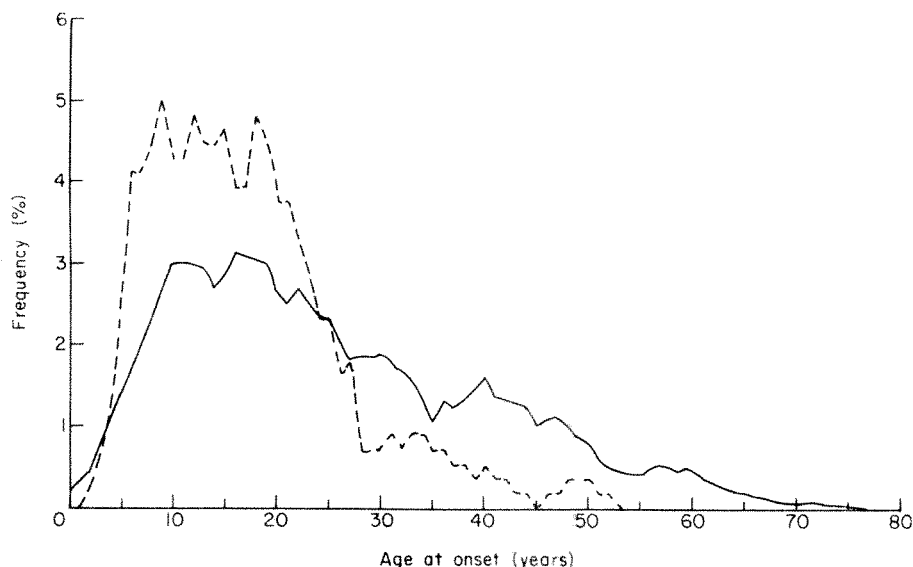


FIGURE 4. Frequency distributions of the age at onset (in 5-year moving averages) for patients with and without a family history of psoriasis. (—) Patients without and (---) patients with family history.

TABLE 3. Mean age at onset $\pm$ standard error of the mean for patients with and without a family history of psoriasis. (The total number of patients in each category is in parentheses)

	No family history	With family history	Difference	Significance (<i>P</i>)
Total sample	25.3 $\pm$ 0.48 (961)	17.2 $\pm$ 0.89 (112)	8.1	< 0.001
Males	29.9 $\pm$ 0.68 (513)	19.9 $\pm$ 1.27 (59)	10.0	< 0.001
Females	20.0 $\pm$ 0.60 (448)	14.2 $\pm$ 1.10 (53)	5.8	< 0.008

Clinical types and sites affected

For clinical and therapeutic convenience the disease was classified into the following groups; extensor psoriasis, flexural psoriasis, psoriasis of the scalp, psoriasis of the nails, palmo-plantar psoriasis, guttate psoriasis, presence of Köbner phenomenon and extensive psoriasis (i.e. when the lesions were diffuse and involved over 50% of the skin surface). 910 (66.6%) had extensor psoriasis, 731 (53.5%) had psoriasis of the scalp, 250 (18.3%) had extensive psoriasis, in 186 (13.6%) the nails were involved, 99 (7.2%) had palmo-plantar psoriasis, 46 (3.2%) had involvement of the flexures, 44 (3.2%) had guttate psoriasis and 25 (1.8%) showed the Köbner phenomenon.

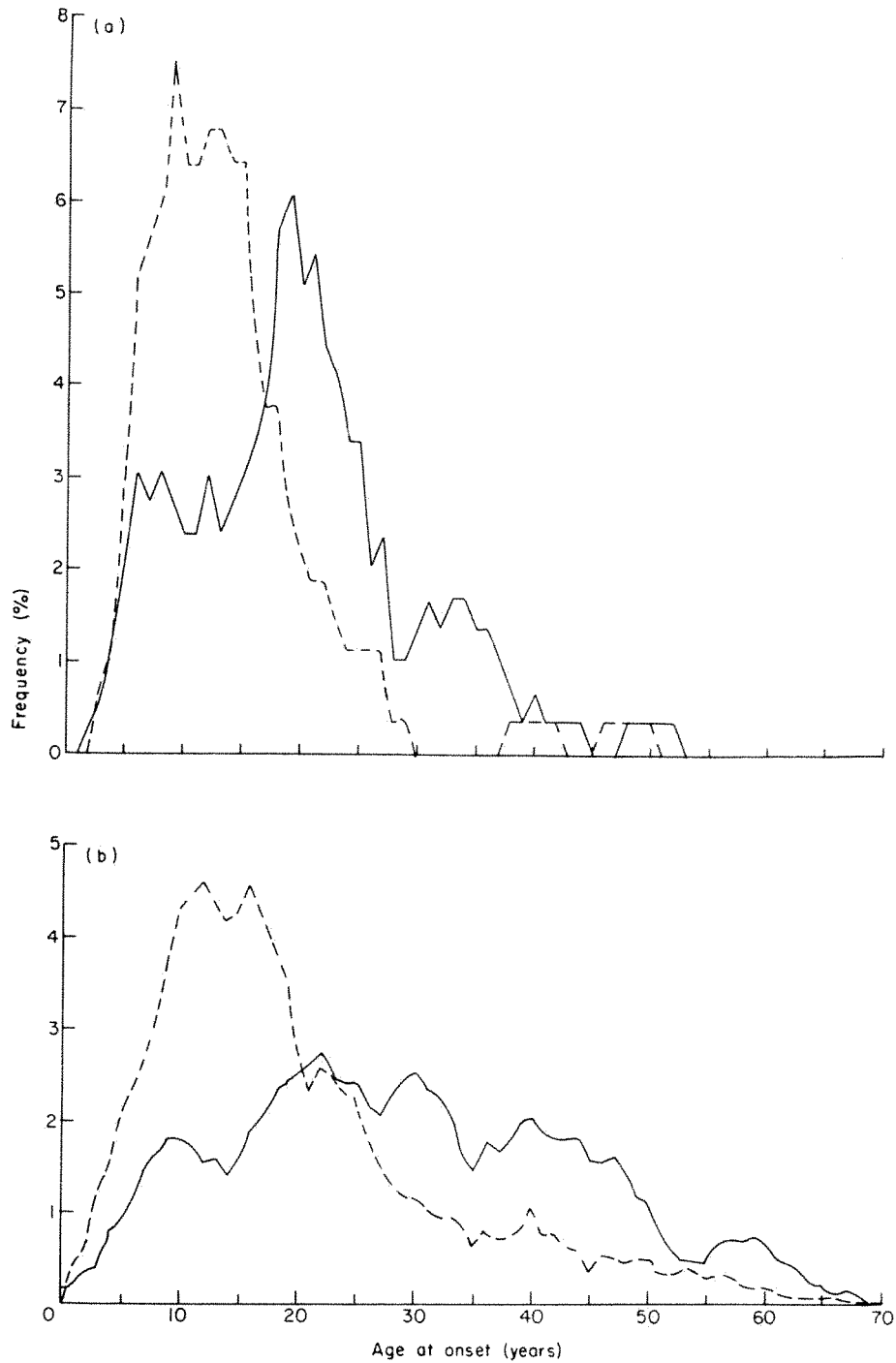


FIGURE 5. Frequency distributions of the age at onset for, (a) males and females with a family history of psoriasis and, (b) for males and females without a family history of psoriasis. (—) Male; (---) female.

There was no significant correlation of these clinical types with age, sex, ethnic group, age at onset of the disease or the presence of a family history.

Precipitating and aggravating factors

74 (5.4%) reported that some factor precipitated the initial or any of the subsequent attacks. In 37 (48.7%) the disease was precipitated by sore throats, in 24 (31.5%) by pregnancy or parturition and in 6 (7.8%) by chloroquine. 33 (89.0%) of patients in whom psoriasis was precipitated by sore throats were under 25 years of age. In the total sample only 51.0% were under 25 years at the onset of the disease. This high proportion with a low age at onset among patients whose disease was precipitated by sore throats is statistically significant ($P < 0.0001$). All patients with chloroquine-induced psoriasis were over 25 years. Sore throats were the commonest precipitating factor in the 5–19 age group and pregnancy and parturition in the 20–34 age group. The other precipitating factors were menarche (2), chicken pox (2), diarrhoea (1), alcohol (1) and mental stress (1). Psychological factors as a precipitating cause, however, were not studied in any great detail. Only one person reported two precipitating factors.

The patients were also questioned as to the time of the year when the disease was worse. Of the 281 patients who were worse during certain months of the year 81 (37.2%) were affected in Nov/Dec, 72 (33.0%) in March/April, 44 (20.1%) in July/August, 23 (10.6%) in Jan/Feb, 19 (8.7%) in May/June and 15 (6.9%) in Sept/Oct. 37 patients reported that their psoriasis was worse during two periods of the year. Fig. 6 shows the mean monthly temperature, rainfall and humidity at Kandy

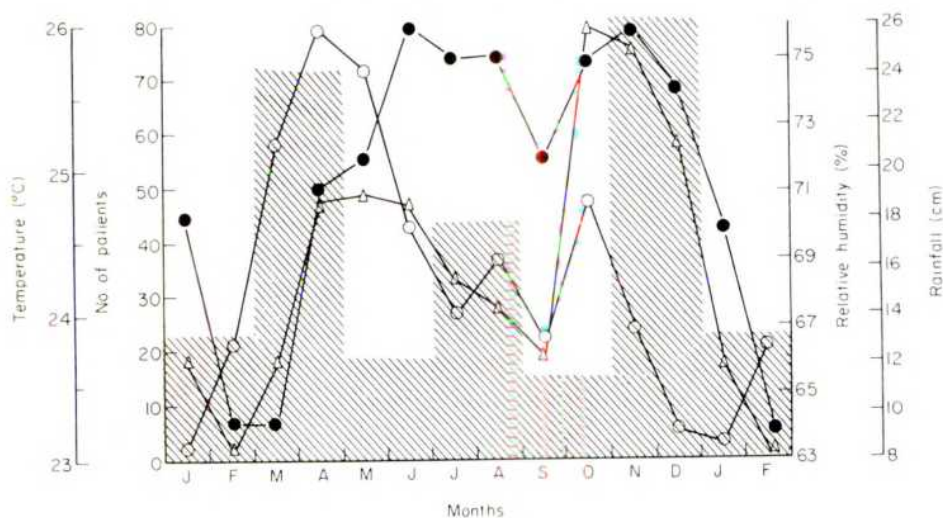


FIGURE 6. Mean monthly temperatures, rainfall and humidity at Kandy, superimposed on a histogram showing the number of patients reporting exacerbations during particular periods of the year. (○) Temperature; (●) relative humidity; (Δ) rainfall.

during the period 1931–1960 (*Statistical Abstracts of Ceylon*, 1969), superimposed on the histogram showing the number of patients whose psoriasis was exacerbated during particular months of the year. A significantly higher proportion of patients reported exacerbations during Nov/Dec and March/April ($P < 0.001$). These peaks of exacerbations coincide with the steeper gradients of temperature, rainfall and humidity (Fig. 6). It appears that the exacerbations of psoriasis are more closely related to the fluctuations rather than to the actual values of temperature, rainfall and humidity.

Of the patients who were affected by the changes in the weather, 159 (72.9%) were under 25 years when they first developed the disease. The corresponding figure for the total sample was 51.0%. The difference is significant at $P < 0.0001$.

Complications

14 (1.0%) had exfoliative erythroderma when first seen by us. 28 (2.0%) had eczematisation of the psoriatic plaques. 73 (5.3%) developed folliculitis which was mainly secondary to treatment with tar. 1240 patients (90.8%) developed no complications and 5 (0.4%) had more than one complication of the disease.

Sixteen patients had sero-negative arthropathy. Only one of them (6.7%) had a family history of psoriasis. Seropositive arthropathy occurred in 5 patients. There was no sex predilection in the patients with arthropathy.

COMMENT

The 1366 patients in this study are the largest consecutive series to be reported from the tropics, comparable in size to the personal series of 1346 patients reported by Ingram (1954). As Lomholt (1971b) observed in Africa, we too find that in the tropics psoriasis in all its essential aspects is like that seen in temperate climates. Generalized pustular psoriasis, however, was not seen perhaps due to the infrequent (1%) use of systemic steroids.

The prevalence rate for psoriasis among white Caucasians has been variously estimated at 1-3% (Gahan, 1943; Ingram, 1954; Baker, 1966; Farber & Nall, 1974). Our estimate for the prevalence rate of psoriasis in Sri-Lanka is 0.4%. This estimate was based on the number of patients seen at the skin clinic in Kandy over a period of 6 years and 4 months. The majority of patients seen at this clinic had major eruptions. Lomholt (1971b) in a census study from the Faroe Islands observed that only 13% of patients with psoriasis had major eruptions. If the distribution of mild and severe psoriasis in Sri-Lanka were similar to that reported by Lomholt, the actual prevalence rate of psoriasis in the population would reach the range quoted for western countries. Our clinic incidence of 8.7% is one of the highest reported.

It is believed that psoriasis is rare among coloured people living in the tropics (Gans, 1952; Ingram, 1954; Baker, Fry & Pegum, 1973). Clinic incidences reported from many tropical countries, which range from 0.3% in Angola (Oliveira, 1957) to 3.2% in Kenya (Verhagen, 1968) tend to support this belief. Our figures for the estimated population prevalence and the clinic incidence point to the contrary. Necessarily a number of factors would have to be considered when the frequency of occurrence of a disease is judged from its clinic incidence. The area covered by the clinic, the mode of referral and the frequency of other diseases seen at the clinic influence the clinic incidence. Other important considerations are the enthusiasm of the patients to seek treatment for psoriasis and the accessibility of the treatment centres. These are in turn dependent on socio-economic factors—a person living under difficult circumstances may not seek treatment for a few patches of psoriasis.

Moreover, in a disease like psoriasis where the patients seek treatment a varying number of years after the onset of the disease, the clinic incidence tends to reflect the population prevalence rather than the population incidence of the disease. The prevalence of a disease is directly proportional to its duration (MacMahon, Pugh & Ipsen, 1960) and for psoriasis the duration of the disease is life-long from the time of onset (Farber, 1971). Therefore, the population prevalence of psoriasis would also be affected by the average life expectancy of the population. Thus the reportedly lower clinic incidences of psoriasis in tropical countries may be related to the lower average life expectancy and the poor socio-economic conditions that prevail in these countries. The remarkable decrease in the clinic

incidence of psoriasis in Germany after the first world war (Gans, 1952) and in Japan after the second world war (Yasuda *et al.*, 1971) may be due to similar reasons. With improved socio-economic conditions and the attendant increase in life expectancy one may expect to see an increase in the prevalence (and the clinic incidence) of psoriasis in the tropical countries even if the population incidence of the disease does not change.

We feel that the prevalence rate of psoriasis is less relevant to the biology of this disease than are its age and sex specific incidence rates. The latter is not subjected to the vagaries of extraneous factors that influence the prevalence rates (and the clinic incidences) of psoriasis. No reliable figures for age and sex specific incidence rates of psoriasis in different populations are available; and admittedly such figures are difficult to collect. However, a useful approximation to these figures could be derived from clinic statistics by calculating the age and sex specific onset rates relative to the population at risk (Burch & Rowell, 1965).

Ingram (1954) in his analysis of the age at onset for 1346 patients found a considerable peak for both males and females during the pubertal years and another minor peak at the climacteric. Based on these data Burch & Rowell (1965) postulated the existence of two distinct but related genetic subpopulations for both sexes; genotype I with an 'early onset' and genotype II with a 'late onset'. We too observed that the frequency distribution curve for the age at onset was bimodal with peaks at the second and fifth decades (Fig. 2). The bimodality of the age at onset is also discernible in some of the frequency distributions presented by Hellgren (1964), Yasuda *et al.* (1971) and Holgate (1975). However, Farber & Nall (1974) did not describe the late onset peak in their series. A late onset peak may escape observation because of a systematic error that nearly always arises in analysing the age at onset (Lomholt, 1971a). If the patients are seen a varying number of years after the onset, it must be expected that a smaller proportion of patients who acquired the disease at a more advanced age will have survived than those who acquired the disease at an earlier age. In the series of Farber & Nall (1974) the difference between the mean age at the time of study and the mean age at onset was 13.3 years. In our series this difference was 4.9 years. This may explain why the late onset peak is not observed in the series by Farber & Nall but is discernible in our series. The use of wide age blocks on a histogram would also tend to submerge minor peaks.

We found that the age at onset was earlier in females and in those with a family history of the disease. The tendency for females to develop the disease earlier than males has been well documented (Hellgren, 1964; Farber & Nall, 1974; Burch & Rowell, 1965; Holgate, 1975). However, it is less often appreciated that patients with a family history of psoriasis also have an earlier age at onset. Asboe-Hansen (1971) in a study of psoriasis in childhood found that two-thirds of children below 16 years were girls and 64% had a family history of psoriasis. Farber & Nall (1974) report that approximately three-fourths of patients with a family history were below 30 years of age when their psoriasis started. Holgate (1975) observed that when one parent had psoriasis 94% of probands of both sexes developed the disease before 30 years, whereas in the total sample only 59.8% developed the disease before 30 years. One of the lowest figures for the median age at onset was reported by Lomholt (1971) from the Faroe Islands—males 13 years and females 12 years. He attributed this to the prevalent environmental factors unfavourable to patients suffering from psoriasis. It is also possible for the low age at onset of psoriasis to be related to the high incidence of family histories among his patients—91% of his patients had a family history, the highest recorded in the literature.

We also found that in patients with an early onset, sore throats often precipitated the disease and that fluctuations of weather frequently caused exacerbations. It is tempting to speculate that females with a family history of psoriasis and a low age at onset form a natural subpopulation. Woodrow *et al.* (1975) found that the histocompatibility antigen HL-A 17 was commoner in female patients and its presence was associated with an earlier age at onset of psoriasis. Evidence for an early age at onset in

HL-A 17 positive psoriatics has also been demonstrated by other workers (White *et al.*, 1972). Thus there appears to be a genetic basis for the subpopulation of females with a low age at onset. This subpopulation may well correspond to the female genotype I postulated by Burch & Rowell. Watson *et al.* (1972) found evidence for multifactorial inheritance of psoriasis. Thus like diabetes mellitus (*British Medical Journal*, 1975), psoriasis too may be a polygenic, genetically heterogeneous disease with several sub-types which differ in their pathogenesis, natural histories and perhaps even in their response to therapy. It is remarkable, however, that the clinical type of the disease did not correlate with the age at onset or with any of the other variables that we studied. Holgate (1975) also found that apart from guttate psoriasis, which occurred before the age of 35 years, there was no significant correlation between the age at onset and the clinical type of the disease.

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REFERENCES

- ASBOE-HANSEN, G. (1971) Psoriasis in childhood. *Proceedings of the International Symposium on Psoriasis, Stanford University*, 1971, pp. 53-59. Stanford University Press, Stanford, California.
- AZULAY, R.D. (1976) Psoriasis in Brazil. *International Psoriasis Bulletin*, **3** (2).
- BAKER, H. (1966) Epidemiological aspects of psoriasis and arthritis. *British Journal of Dermatology*, **78**, 249.
- BAKER, H., FRY, L. & PEGUM, J.S. (1973) Psoriasis. In: *Recent Advances in Dermatology* (ed. by A. Rook), p. 425. Churchill Livingstone, Edinburgh & London.
- BRITISH MEDICAL JOURNAL (1975) Leading article. Heritability of diabetes mellitus. *British Medical Journal*, **iv**, 127.
- BURCH, P.R.J. & ROWELL, N.R. (1965) Psoriasis: Aetiological aspects. *Acta dermato-venereologica*, **45**, 366.
- FARBER, E.M. & COX, A.J. (1967) The biology of psoriasis. *Journal of Investigative Dermatology*, **49**, 348.
- FARBER, E.M. (1971) Studies of the nature and management of psoriasis. *California Medicine*, **114**, 1.
- FARBER, E.M. & NALL, M.L. (1974) Natural history of psoriasis in 5600 patients. *Dermatologica*, **148**, 1.
- GAHAN, E. (1943) Incidence of psoriasis among the population at large. *Archives of Dermatology and Syphilology*, **43**, 305.
- GANS, O. (1952) Some observations on the pathogenesis of psoriasis. *Archives of Dermatology and Syphilology*, **66**, 598.
- HELLGREN, L. (1964) Psoriasis: a statistical, clinical and laboratory investigation of 255 psoriatics and matched healthy controls. *Acta dermato-venereologica*, **44**, 191.
- HOLGATE, M.C. (1975) The age of onset of psoriasis and the relationship to parental psoriasis. *British Journal of Dermatology*, **92**, 443.
- INGRAM, J.T. (1954) The significance and management of psoriasis. *British Medical Journal*, **ii**, 823.
- KERDAL-VEGAS, F. (1971) Psoriasis in South America. Geographic and Racial factors. *Proceedings of the International Symposium on Psoriasis, Stanford University*, 1971, pp. 35-39. Stanford University Press, Stanford, California.
- KHALEQUE, K.A. & BASIT, A. (1968) Study of psoriasis cases in East Pakistan. *Journal of Tropical Medicine and Hygiene*, **71**, 20.
- LOMHOLT, G. (1963) Psoriasis: prevalence, spontaneous course and genetics. Copenhagen, GEC Gad. Diss.; cited by Farber & Nall (1974).
- LOMHOLT, G. (1971a) Environment and genetics in psoriasis. *Proceedings of the International Symposium on Psoriasis, Stanford University*, 1971, pp. 21-24. Stanford University Press, Stanford, California.

- LOMHOLT, G. (1971b) Psoriasis in Uganda: a comparative study with other parts of Africa. *Proceedings of the International Symposium on Psoriasis, Stanford University, 1971*, pp. 41-48. Stanford University Press, Stanford, California.
- LUDOWYKE, E.F.C. (1962) *Short story of Ceylon*, p. 16. Faber & Faber, London.
- MACMAHON, B., PUGH, T.F. & IPSEN, J. (1960) *Epidemiologic methods*, pp. 60-61. Little, Brown & Co., Boston.
- MASAWA, A.E.J. (1973) Psoriasis in Tanzania. *International Psoriasis Bulletin*, 1 (2).
- OLIVEIRA, A. DE (1957) Notes on dermatology and venereology in two towns of Angola (Lobito and Benguela: 1954-1957). *Journal do Medico (Lisbon)*, 34, 597; cited by Lomholt (1971b).
- Statistical Abstracts of Ceylon* (1969) Department of Census and Statistics Department of Government Printing, Colombo, 1970.
- VERHAGEN, A.R.H.B. & KOTEN, J.W. (1967) Psoriasis in Kenya. *Archives of Dermatology*, 96, 39.
- VERHAGEN, A.R.H.B., KOTEN, J.W., CHADDAH, V.K. & PATEL, R.I. (1968) Skin diseases in Kenya. *Archives of Dermatology*, 98, 577.
- WATSON, W., HOWARD, M.C., FARBER, E.M. & HALL, M.L. (1972) The genetics of psoriasis. *Archives of Dermatology*, 105, 197.
- WHITE, S.H., NEWCOMER, V.D., MICKEY, M.R. & TERASAKI, P.I. (1972) Disturbance of HL-A antigen frequency in psoriasis. *New England Journal of Medicine*, 287, 740.
- WOODROW, J.C., DAVE, V.K., USHER, N. & ANDERSON, J. (1975) The HL-A system and psoriasis. *British Journal of Dermatology*, 92, 427.
- YASUDA, T., ISCHIKAWA, E. & MORI, S. (1971) Psoriasis in the Japanese. *Proceedings of the International Symposium on Psoriasis. Stanford University, 1971*, pp. 25-34. Stanford University Press, Stanford, California.

Lymphocytotoxicity in mycosis fungoides

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SUMMARY

The phenomenon of lymphocytotoxicity was demonstrated in 29 patients with mycosis fungoides, using a newly described epithelial cell culture system and an isotope marker, ^{125} Iododeoxyuridine (^{125} IUdR). Lymphocytotoxicity was demonstrated in patients in the generalized plaque and the erythroderma phases of the disease ($P \leq 0.05$). No significant lymphocytotoxicity was demonstrable in patients who were in the limited plaque (early) phase of the disease. It is hypothesized that this demonstration of lymphocytotoxicity in patients may be a manifestation of a delayed hypersensitivity response to an as yet unidentified antigen in the epidermis.

That there is an immunological reaction to various skin diseases has been observed on numerous occasions. Pinkus, Jallad, & Mehregan (1963) and Payan, Gilbert & Jacobs (1970) commented on the inflammatory infiltrate in metastatic and precancerous lesions. Others have commented on the immunological reactions seen in contact dermatitis (Jordan & Finn, 1974; Jones, Reinhardt & Rinaldi, 1974) and in lichen planus-like graft versus host reactions (Saurat *et al.*, 1975). The presence of lymphocytotoxicity has been described in recurrent aphthous stomatitis (Rogers, Sams & Shorter, 1974), and in malignant melanoma and halo naevi (Roenigk *et al.*, 1975).

Mycosis fungoides has recently been categorized as a T cell disease (Lutzner, Edelson & Schein, 1975). While its clinical course is fairly well described, the aetiology and the immunological aspects of the disease are still not clearly understood. Nordqvist & Kinney (1976) and MacKie *et al.* (1976), described the finding of decreased T cells and elevated null cells in patients with late stage mycosis fungoides. Ryan *et al.* (1973) raised the hypothesis that mycosis fungoides begins in the epidermis. This hypothesis could correlate with the histological appearance of early stage mycosis fungoides in which infiltrates of atypical lymphocytes present in the dermis obscure the dermal-epidermal junction and are also found within the epidermis both singly and in aggregates (Pautrier's microabscesses) (Indianer, 1973).

Our study was done to determine whether there was any immunological reaction (lymphocytotoxicity) between the epidermal cells and the serum and lymphocytes of patients with mycosis fungoides. The concept that there is recognition of neoplastic changes by the immunological system of the body

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has intrigued investigators since the turn of the century. To date many animal neoplasms have been shown to contain tumour specific transplantation antigens (Hellstrom & Hellstrom, 1969). The colony inhibition assay was developed to demonstrate these antigens (Hellstrom *et al.*, 1968; Hellstrom *et al.*, 1969). An adaptation of Karasek & Charlton's (1971) method for isolating and plating pure epidermal cells onto collagen gel medium was used to evaluate psoriasis vulgaris for disease specific antigens using the collagen inhibition technique (Miller & Harris, unpublished data) and is used in this report which describes the presence of lymphocytotoxicity in mycosis fungoides.

METHODS

Dermatome shave sections of skin were taken from 29 patients with mycosis fungoides, prior to having received any form of therapy, who were in the limited plaque (early) phase, in the generalized plaque (late) phase, or in the erythrodermic phase of the disease. Specimens were taken from lesions and from the normal appearing uninvolved skin, except in those patients who were erythrodermic. The specimens were immediately placed into 10 ml of Hanks' balanced salt solution containing 200 units of penicillin and streptomycin and was incubated for approximately 30 min at 37°C. The specimens were then cut into 1 cm square pieces and incubated in prewarmed 0.3% trypsin for 30 min, following which the pieces were rinsed three times in 0.9% saline solution and left in final wash for 20 min. The excess saline was then removed, and the specimens drained and transferred to Petri dishes containing 5 ml of MEM with 10% fetal calf serum, penicillin and streptomycin (100 units per mixture). The dermis and epidermis of the specimens were then separated with a pair of No. 10 Bard-Parker scalpels. Viable epidermal cells were released into the growth medium from the undersurface of the epidermis. This was done by gently scraping the undersurface with the blades. Fragments of dermis and epidermis were removed and discarded. The liberated cells were dispersed in the medium, counted and plated (at a concentration of 5×10^4 cells per well) onto Microtest II plates which were collagen coated.

Extraction of lymphocytes and monocytes from peripheral blood

These cells were harvested from 20 ml of blood by a modification of the Ficoll gradient technique (Throsby & Bratlie, 1970). Defibrinated blood was diluted to 2:1 with Hanks' balanced salt solution, layered on an Isopaque-Ficoll gradient (ratio 10:24), and centrifuged at 1600 rev/min for 35 min. The WBC suspension was passed through packed Fenway nylon to extract granulocytes and then washed in Hanks' Balanced Salt Solution. The suspension of approximately 90% lymphocytes was diluted to 5×10^5 cells/ml. The lymphocytes and monocytes were then utilized in the various procedures described herein.

LYMPHOCYTOTOXICITY PROCEDURE

The epidermal cells in the wells were divided into four sections: (a) wells receiving epidermal cells only (E); (b) wells receiving epidermal cells and lymphocytes (E + L); (c) wells receiving epidermal cells and serum (E + S); and (d) wells receiving epidermal cells, lymphocytes and serum (E + L + S). Epidermal cells were plated to each well at a concentration of 5×10^4 cells per well. The volume of each well was adjusted to 125 μ l with medium. These were incubated for 24 h, the medium removed, and the cells labelled with 1 μ Ci per well of ^{125} iododeoxyuridine (^{125} IUdR) for 8 h. They were then washed twice with medium and lymphocytes added to the designated wells at a concentration of 5×10^5 lymphocytes per well. Serum from each patient (heat inactivated at 56°C) diluted 1:5 with MEM was added to the designated wells. The plate was then incubated for a period of 48–72 h after

which 25 μ l of the supernatant was collected for isotope count to measure the ^{125} IUdR released. Each determination was performed in quadruplicate.

TABLE 1. Results of lymphocytotoxicity tests with skin from diseased and uninvolved areas

	Diseased skin				Normal skin			
	E	E+L	E+L+S	E+S	E	E+L	E+L+S	E+S
Limited plaque (early) phase	1.00	1.30	1.51	2.07	1.00	1.03	0.98	1.23
	1.00	1.06	1.13	1.33	1.00	1.16	1.16	1.15
	1.00	0.36	0.87	0.35	1.00	0.81	0.93	0.97
	1.00	0.47	0.65	3.39	1.00	1.24	0.87	—
	1.00	1.00	1.19	1.54	1.00	1.49	1.06	1.27
	1.00	1.44	2.10	1.80	1.00	1.11	0.47	1.00
	1.00	0.97	0.75	1.30	1.00	0.93	0.97	1.08
	1.00	0.69	1.15	0.97	1.00	1.46	1.46	1.70
	1.00	0.73	0.66	0.63	1.00	1.02	0.99	0.91
	1.00	1.19	0.94	1.21	1.00	1.15	0.98	1.39
	1.00	1.12	0.84	1.26	1.00	2.06	1.80	3.55
	1.00	1.35	1.49	1.38	1.00	0.99	0.97	1.02
	1.00	1.11	1.03	0.93	1.00	1.02	1.05	0.90
Mean	1.00	0.98	1.10	1.39	1.00	1.19	1.05	1.34
Generalized plaque (late) phase	1.00	1.60	1.44	1.94	1.00	0.57	0.73	0.54
	1.00	0.96	1.07	1.36	1.00	0.83	0.73	1.04
	1.00	1.14	1.21	1.19	—	—	—	—
	1.00	1.24	1.37	1.47	—	—	—	—
	1.00	0.94	0.96	1.29	1.00	0.99	0.91	1.18
	1.00	0.97	—	0.37	1.00	0.97	0.69	0.67
	1.00	0.93	0.78	0.91	1.00	0.95	1.76	0.80
	1.00	1.01	1.25	0.50	1.00	1.16	1.23	1.37
	1.00	1.04	0.90	1.06	1.00	0.47	0.59	0.63
	1.00	0.86	0.88	1.30	1.00	1.01	0.96	1.01
Mean	1.00	1.06	1.09	1.00	1.00	0.86	0.82	0.90
Erythroderma phase	1.00	1.11	0.88	1.03	—	—	—	—
	1.00	0.97	0.97	1.09	—	—	—	—
	1.00	1.26	0.89	1.55	—	—	—	—
	1.00	2.35	2.33	1.21	—	—	—	—
	1.00	1.55	1.67	1.46	—	—	—	—
	1.00	5.01	7.21	1.01	—	—	—	—
Mean	1.00	2.04	2.32	1.22	—	—	—	—
Mean (overall)	1.00	1.23	1.36	1.27	1.00	1.06	0.96	1.17

LYMPHOCYTOTOXICITY RESULTS

The isotope counts were given in counts per minute for each 25 μ l sample per well. The ratio for isotope counts of each well was then calculated with the well containing epidermal cells only given an index number of one in order to 'normalize' the results from individual patient's experiments for computation purposes. The mean, standard deviation, standard error and *P* values were obtained for each patient's results, by analysis using the Kruskal-Wallis test statistic (Dixon, 1975). Tabulations

TABLE 2. Statistical correlations of results from patient groupings

Patient group	MF vs MF + L	MF vs MF + L + S	MF vs MF + S	MF + L vs N + L	MF + L + S vs N + L + S	MF + S vs N + S
Whole group	0.1450	0.6238	0.0003*	0.8956	0.3313	0.5327
Limited plaque	0.4682	0.7217	0.749	0.2814	1.0000	0.6649
Generalized plaque	0.225	0.6712	0.1062	0.0311*	0.0403*	0.4623
Erythroderma group	0.0403*	1.0000	0.0021*	—	—	—

* $P < 0.05$.

are listed in Table 1. The clinical state of the patients' disease was compared to the findings of the lymphocytotoxicity tests done on the patients' lesions. None was observed to have enlarged lymph nodes or internal involvement.

Statistical correlations between the results obtained from various wells are listed in Table 2.

The results show that there is a significant difference in the degree of lymphocytotoxicity exhibited against the diseased skin of patients in the generalized plaque and erythroderma phases of the disease. Patients in the generalized phase had a significant increase in the amount of $^{125}\text{IUdR}$ released from epidermal cells of the diseased skin when a comparison was made to uninvolved skin epidermal cells with lymphocytes only and with lymphocytes and serum added to the system. In patients with erythroderma there was an increased release of $^{125}\text{IUdR}$ epidermal cells when lymphocytes only were added and when serum only was added. There was no significant increase in the release of $^{125}\text{IUdR}$ isotope from the wells in which epidermal cells from patients in the limited plaque phase of the disease were cultured.

DISCUSSION

These findings would suggest that there is an 'enhancing' phenomenon occurring in the immunological interrelationship between the lymphocyte, serum and the diseased skin in the patients in the generalized and erythroderma phase of the disease, while there is no apparent change in patients in the limited plaque phase of the disease.

There are many technical problems associated with this form of investigation. The major difficulty is the relative scarcity of patients with mycosis fungoides. At our Mycosis Fungoides Clinic in the Stanford Medical Center there are approximately only 25 new patients a year who present themselves with the disease and it is not always possible to obtain specimens from all these patients in order to carry out the studies mentioned herein. Obtaining enough skin for sufficient numbers of microcultures has only recently been a practicality since the collagen gel technique was described by Karasek (1971). The problem of finding a suitable isotope-marker for measuring lymphocytotoxicity in the cultured epidermal cells has proved to be a major difficulty: the phenomenon of lymphocytotoxicity has been demonstrated in other systems by the use of $^{51}\text{chromium}$ and ^3H thymidine. However, we did not find these to be of sufficient reliability and practicality in our preliminary studies and we subsequently decided on the use of $^{125}\text{IUdR}$. We could find no description of the previous use of this isotope for measuring lymphocytotoxicity in cutaneous diseases. $^{125}\text{IUdR}$ has been used to measure tumour immunity in Moloney sarcomas and B16 melanoma of mice (Seeger & Owen, 1973; Kreider *et al.*, 1974). It is particularly useful in that it remains within the cells for a period of days, in comparison to $^{51}\text{chromium}$ which is spontaneously released from cells within hours. The retention

of the $^{125}\text{IUdR}$ within the cells for a long period is ideal for epidermal cell cultures which are done over a time span of a week.

The problem of non-specific spontaneous release of isotope from epidermal cells is difficult to control. For this reason all experiments had control wells of epidermal cells cultured alone and indexing was done to allow for non-specific spontaneous release of the isotope. The use of $^{125}\text{IUdR}$ is relatively new and each experiment in which it is used requires that optimal labelling and incubation times with the isotope be evaluated. Ting *et al.* (1975) have recently shown that a 20 h incubation time instead of a longer period may be utilized to avoid a high non-specific release of isotope; however, their study was done in laboratory animals and with a laboratory cultured virus-induced mouse leukaemia. This shorter incubation period may be applicable to our methods using human tissue.

Our laboratory methods did not utilize cells from other patients; this was done to avoid non-specific cytotoxicity against non-autologous tissues. Furthermore, our method was designed to study the differences in lymphocytotoxicity when lymphocytes and serum, or both, were added to epidermal cells. There was thus no need to obtain 100% lysis or complete lymphocytotoxicity by using lymphocytes from other patients for controls in our method.

These results raise some interesting speculations into the nature of mycosis fungoides. Should our data prove to be reproducible in further studies on lymphocytotoxicity and also on lymphoblastogenesis and migration inhibition (which we are presently undertaking), one could conclude that in the early limited plaque phase of the disease the host immune system does not mount a delayed type of hypersensitivity reaction against the diseased areas in skin, while in the later generalized plaque phase of the disease and also in erythrodermic mycosis fungoides the host immune system is probably altered and able to mount such a reaction. It would be interesting to study the responses of patients' tissue in the tumour phase of the disease.

The presence of an infiltrate of lymphocytes within the epidermis is seen in several diseases: allergic contact dermatitis, pityriasis lichenoides, lichen planus, syphilis, erythema multiforme, drug eruptions and, when the lymphocytes have an atypical morphology, mycosis fungoides, lymphomatoid papulosis and histiocytosis X (Lever, 1967). In allergic contact dermatitis and drug eruptions, the lymphocytes are attracted to the epidermis by the antigen formed by a hapten-protein combination through the mechanism of a delayed hypersensitivity reaction. Lymphocytes may also be attracted to the epidermis by a similar mechanism in the other listed diseases. Silberberg, Baer & Rosenthal (1976) described the possible role of Langerhans cells within the epidermis in allergic contact dermatitis as being involved in the delayed hypersensitivity reaction. Granules similar to these of Langerhans cells have also been described in histiocytosis X (Niebauer, Krawczyk & Wilgram, 1970). Ryan *et al.* (1973) proposed that the Langerhans cell may be the cell responsible for the disease process. Tan *et al.* (1974) suggested that mycosis fungoides may be a disease of antigen persistence resulting in an immunological imbalance and eventuating into an auto-immune disease or a malignancy. Rowden & Lewis (1976) have recently suggested that the Langerhans cell may be involved in the pathogenesis of mycosis fungoides.

The presence of atypical lymphocytes within the epidermis in mycosis fungoides may be the result of a delayed hypersensitivity response. Our data may be a demonstration of this phenomenon. However, while this may be evidence in support of the hypothesis that there is a factor within the epidermis attracting the atypical lymphocytes, further investigations are necessary, and the patients studied in this paper will be followed closely to determine whether there are any changes with the passage of time.

REFERENCES

- DIXON, W.J. (1975) *Biomedical Computer Programs*. University of California Press, Los Angeles.
- HELLSTROM, E. & HELLSTROM, I. (1969) Cellular immunity against tumor antigens. *Advances in Cancer Research*, **12**, 167.
- HELLSTROM, I., HELLSTROM, K.E., EVANS, C.A. *et al.* (1969) Serum-mediated protection of neoplastic cells from inhibition by lymphocytes immune to their tumor-specific antigens. *Proceedings of the National Academy of Sciences of the United States of America*, **62**, 362.
- HELLSTROM, I., HELLSTROM, K.E., PIERCE, G. *et al.* (1968) Cellular and humoral immunity to different types of human neoplasms. *Nature*, **220**, 1352.
- INDIANER, L. (1973) Histopathology of mycosis fungoides. *Cutis*, **11**, 307.
- JONES, E.H., REINHARDT, J.H. & RINALDI, M.G. (1974) Acquired immunity to dermatophytes. *Archives of Dermatology*, **109**, 840.
- JORDAN, W.I. & FINN, J.W. (1974) Flare dermatitis. *Archives of Dermatology*, **109**, 884.
- KARASEK, M.A. & CHARLTON, M.E. (1971) Growth of post-embryonic skin epithelial cells on collagen gels. *Journal of Investigative Dermatology*, **56**, 205.
- KRIEDER, J.W., FLICKINGER, J.T., LENGLE, N. *et al.* (1974) A lymphocytotoxicity assay not requiring the *in vitro* culture of target tumor cells. *Transplantation*, **18**, 223.
- LEVER, W.F. (1967) *Histopathology of the Skin*, 4th edn. Lippincott, Philadelphia, Pennsylvania.
- LUTZNER, M., EDELSON, R. & SCHEIN, P. (1975) Cutaneous T-cell lymphomas. *Annals of Internal Medicine*, **83**, 534.
- MACKIE, R., SLESS, F.R., COCHRAN, R. & DESOUSA, M. (1976) Lymphocyte abnormalities in mycosis fungoides. *British Journal of Dermatology*, **94**, 173.
- MILLER, K. & HARRIS, D. (1977) Lymphocytotoxicity in psoriasis. Unpublished paper.
- NEUBAUER, G., KRAWCZYK, W. & WILGRAM, G.F. (1970) Langerhans cell organelles in Letterer-Siwe. *Archiv für Klinische und Experimentelle Dermatologie*, **23**, 125.
- NORDQVIST, B.C. & KINNEY, J.P. (1976) T and B cells and cell-mediated immunity in mycosis fungoides. *Cancer*, **37**, 714.
- PAYAN, H.M., GILBERT, E.F. & JACOBS, W.H. (1970) Lymphocytic reaction around primary and metastatic melanomas. *Southern Medical Journal*, **63**, 1350.
- PINKUS, H., JALLAD, M. & MEGREGAN, A.H. (1963) The inflammatory infiltrate of precancerous skin lesions. *Journal of Investigative Dermatology*, **40**, 247.
- ROENIGK, H.H., DEODHAR, S.D., KREBS, J.A. *et al.* (1975) Microcytotoxicity and serum blocking factors in malignant melanoma and halo nevus. *Archives of Dermatology*, **111**, 720.
- ROGERS, R.S., SAMS, W.M. & SHORTER, E.G. (1974) Lymphocytotoxicity in recurrent aphthous stomatitis. *Archives of Dermatology*, **109**, 361.
- ROWDEN, G. & LEWIS, M.G. (1976) Langerhans cells: Involvement in the pathogenesis of mycosis fungoides. *British Journal of Dermatology*, **95**, 665.
- RYAN, E.A., SANDERSON, K.V., BARTAK, P. & SAMMAN, P.D. (1973) Can mycosis fungoides begin in the epidermis? A hypothesis. *British Journal of Dermatology*, **88**, 419.
- SAURAT, J.H., GLUCKMAN, E., BUSSEL, A. *et al.* (1975) The lichen planus-like eruption after bone marrow transplantation. *British Journal of Dermatology*, **92**, 675.
- SEEGER, R.C. & OWEN, J.J.T. (1973) Measurement of tumour immunity *in vitro* with ¹²⁵Iododeoxyuridine-labelled target cells. *Transplantation*, **15**, 404.
- SILBERBERG, I., BAER, R.L. & ROSENTHAL, S.A. (1976) The role of Langerhans cells in allergic contact hypersensitivity. *Journal of Investigative Dermatology*, **66**, 210.
- TAN, R.S., BUTTERWORTH, C.M., McLoughlin, H. *et al.* (1974) Mycosis fungoides—a disease of antigen persistence. *British Journal of Dermatology*, **91**, 607.
- TING, C.C., BUSHAR, G.S., RODRIGUES, D. *et al.* (1975) Cell-mediated immunity to friend virus-induced leukemia. *Journal of Immunology*, **115**, 1351.
- THROSBY, E. & BRATLIE, A. (1970) A rapid method for preparation of pure lymphocyte suspensions. In: *Histo-compatibility Testing* 1970. (ed. by P. Terasaki), p. 655. Williams and Wilkins Co., Baltimore, Maryland.

Case Reports

Penicillamine pemphigus and the nephrotic syndrome occurring simultaneously

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SUMMARY

Proteinuria and the nephrotic syndrome are well known to occur as complications of penicillamine therapy, but reports of penicillamine-induced pemphigus have been relatively few. This is the report of a case in which both complications occurred simultaneously.

CASE REPORT

A 48-year-old Caucasian male had had severe seropositive rheumatoid arthritis for 6 years. There was no previous history of renal disease and urea, creatinine and serum proteins were within normal limits. ESR was 20 mm/h. D-Penicillamine therapy was begun at 250 mg daily, increasing over the course of 3 months to 1000 mg daily. This dose was maintained without significant clinical improvement until after 1 year he developed multiple red scaly macules on the trunk and scalp, which coalesced over the course of the next 6 weeks to involve large areas. Small flaccid blisters were present, and weeping erosions. The oral mucosa was not involved. Over the same 6 weeks his ankles, face and scrotum had become oedematous and heavy proteinuria had developed.

Investigations

Serum albumin 22g/l, urea 8 mmol/l, creatinine 0.08 mmol/l, cholesterol 11.5 mmol/l, ESR 57 mm/h. The 24 h urinary protein was 19 g. Urine protein electrophoresis showed a clearance ratio of 0.05, indicating a selective proteinuria. Antinuclear factor was negative. Levels of immunoglobulins, C3 and C4 were normal.

Biopsy of involved skin showed a high intraepidermal cleft with acantholysis. Some parts of the epidermal basement membrane showed liquefactive degeneration, and there was a perivascular infiltrate of lymphocytes. The histology was compatible with pemphigus foliaceus, with some features of pemphigus erythematosus. Direct immunofluorescence on lesional skin showed IgG in the epidermal intercellular area. There was no basement membrane staining. Serum contained antibody to epidermal intercellular cement to a titre of 1/320. Tests for circulating antibodies to penicillamine were performed, using a method involving enzyme release from neutrophils (Watson *et al.*, 1977) and were positive.

A diagnosis of pemphigus foliaceus and nephrotic syndrome due to penicillamine was made. He was treated with prednisone, diuretics and protein replacement, but the skin involvement initially

became more widespread with profuse fluid exudation. He developed a deep vein thrombosis and was anticoagulated. Prednisone 90 mg daily controlled the blistering but he then had melaena from gastric erosions. Other complications attributed to corticosteroids were generalized herpes zoster, oral candidiasis and diabetes mellitus.

Two months after penicillamine therapy had ceased proteinuria had fallen to 5 g per day, and the serum creatinine remained within the normal range. Prednisone in high dosage was still necessary to control the pemphigus. Azathioprine 100 mg daily was added but had to be withdrawn shortly afterwards as he developed several subcutaneous abscesses, followed by septicaemia and bronchopneumonia due to *Proteus mirabilis* and *Pseudomonas aeruginosa*. He died 3 months after admission. Post mortem examination revealed *Aspergillus fumigatus* infection of the lungs, brain and other organs. Immunofluorescent studies of the kidney showed heavy fine granular deposits of IgG and C₃ on the glomerular basement membrane.

DISCUSSION

Penicillamine has proved to be a significant advance in the management of rheumatoid disease but is justly given with caution in view of its side effects. Among them, pemphigus is now well recognized by dermatologists (Hewitt, Benveniste & Lessana-Leibovitch 1975; Marsden *et al.*, 1976) and is probably more common than the approximately 25 reported cases would suggest. This patient had the appearances of pemphigus foliaceus progressing to extensive exuding erosions, but without mucous membrane involvement. The degeneration of the epidermal basement membrane was suggestive of pemphigus erythematosis, but ANF was negative and there was no staining of the basement membrane area on direct immunofluorescence. Renal histology was not that of L.E. However, some reported cases of penicillamine allergy have had ANF or L.E. cells and one had immunofluorescent features of both pemphigus and L.E. (Kennedy, 1977).

In addition to fixed and circulating antibody to intercellular cement substance (ICS antibody) in fairly high titre, this patient had circulating antibodies to penicillamine. It seems likely that the ICS antibody is directly pathogenic to the skin and this view is supported by the findings of Benveniste *et al.* (1975) that all of 5 patients with penicillamine-pemphigus had ICS antibody, whereas 40 patients on penicillamine without pemphigus did not have it.

The fatal outcome of this case indicates that penicillamine-induced pemphigus, although often resolving on stopping the drug, may require high and prolonged steroid dosage for its control, and the resulting complications (gastric haemorrhage, viral, bacterial and fungal superinfection in the patient) may result in high morbidity and a mortality similar to pemphigus vulgaris.

Renal biopsy studies by Bacon *et al.* (1976) have shown that penicillamine nephropathy is due to the deposition of immune complexes containing IgG and C₃ on the glomerular basement membrane, with minimal change on light microscopy. These deposits may persist for at least a year after stopping penicillamine and may result in permanent renal damage resembling idiopathic membranous nephropathy. They imply that penicillamine nephropathy should be regarded more seriously than hitherto.

This case also raises the possibility that the renal manifestations are due to the deposition of complexes of penicillamine-ICS antibody, or penicillamine-antipenicillamine antibody, since both antibodies were present in the circulation. Further studies are necessary to see whether ICS antibody is present in penicillamine treated patients before pemphigus occurs and whether it is present in those with proteinuria.

ACKNOWLEDGMENTS

I thank Dr E.L.Rhodes for permission to report this case and Dr B.Watson for performing the test for penicillamine antibodies.

REFERENCES

- BACON, P.A., TRIBE, C.R., MACKENZIE, J.C., VERRIER JONES, J., CUMMING, R.H. & AMER, B. (1976) Penicillamine nephropathy in rheumatoid arthritis. *Quarterly Journal of Medicine*, **45**, 661.
- BENVENISTE, M., CROUZET, J., HOMBERG, J.C., LESSANA, M., CAMUS, J.P. & HEWITT, J. (1975) Pemphigus induits par la D-penicillamine dans la polyarthrite rhumatoïde. *La Nouvelle Presse Medicale*, **4**, 3125.
- HEWITT, J., BENVENISTE, M. & LESSANA-LEIBOWITCH, M. (1975) Pemphigus induced by D-penicillamine. *British Journal of Dermatology*, **93**, Suppl. **11**, 12.
- KENNEDY, C. (1977) Personal communication.
- MARSDEN, R.A., RYAN, T.J., VANHEGAN, R.I., WALSH, M., HILL, H. & MOWAT, A.G. (1976) Pemphigus foliaceus induced by penicillamine. *British Medical Journal*, **iv**, 1423.
- WATSON, B., GANGE, R.W., RHODES, E.L.R. & ROSLING, A.E. (1977) The release of B glucuronidase from leukocytes in the presence of immune complexes. *British Journal of Dermatology*, **96**, 349.

Dermatitis rhabditidosa in an 11-year-old girl

A NEW CUTANEOUS PARASITIC DISEASE OF MAN

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SUMMARY

Rhabditiform larvae of *Rhabditis* (*Pelodera*) *strongyloides* caused pruritic lesions in an 11-year-old girl, and persisted for 2½ months. Larvae were found in skin scrapings from the child and in the family dog's hair.

The larvae of various nematodes can be the cause in man of skin lesions, mostly very pruritic, papulo-vesicular in character and of varying duration (Table 1). *Rhabditis strongyloides* A. Schneider, 1866 (*Pelodera strongyloides*, see Thorne, 1961) is a free-living nematode found both in soil rich in organic substances and in cattle and horse manure and sheep faeces. The larvae of this nematode can be found on the skin of dogs, cattle and horses, and probably feed on desquamated epidermis of the host; in these conditions they do not attain sexual maturity (Stefański, 1959; Stefański & Zarnowski, 1971). The larvae can cause extensive baldness on the limbs, sides of the neck, hypogastric area, and lateral aspects of the body, a decrease in the elasticity of the skin, and itching. These symptoms in animals are termed dermatitis rhabditidosa or verminosa (Hellebrand, 1924; Chitwood, 1932; Hayes, 1953; Rhode *et al.*, 1953; Ślusarski *et al.*, 1956; Cheng, 1964; Muller & Kirk, 1969).

The purpose of this paper is to present observations on the pathology of the dermatitis rhabditidosa in a child, caused by an invasion of larvae of the nematode *Rhabditis strongyloides* not, to our knowledge, previously described in the literature.

CASE REPORT

An 11-year-old girl was examined in the Institute of Paediatrics because of pruritic lesions on the skin of the trunk and lower limbs. These had erupted within a few days a month previously and did not disappear despite treatment with crotamiton ointment. No one else in the family had any skin lesions. In the family dog, however, which the child always played with and often took to bed, minor thinning of the hair on the abdomen had been observed and the dog had suddenly died a week previously. On the day of the child's consultation the skin on the trunk, especially the abdomen and buttocks, the lateral and anterior parts of the thighs and legs, as well as the left arm exhibited profuse

TABLE 1. Larvae of some nematodes causing cutaneous disease*

Type	Infection from	Larvae	Clinical characteristics	Name	Duration
<i>Necator americanus</i>	Human	Filariform	Papulovesicular; pruritic; minimal migration in skin	Hookworm disease;	2 weeks
<i>Ancylostoma duodenale</i>	Human	Filariform	Papulovesicular; pruritic; minimal migration in skin	Uncinarial dermatitis 'ground itch' or 'dew itch'	2 weeks
<i>Ancylostoma braziliense</i>	Cat, dog	Filariform	Thread-like, pruritic, migratory burrow; moving 1 or 2 cm/day		Several weeks or months
<i>Ancylostoma caninum</i>	Dog	Filariform	Papular, rarely linear lesions	Cutaneous larva migrans; creeping eruption	2 weeks
<i>Uncinaria stenocephala</i>	European dog	Filariform	Thread-like, pruritic migratory burrow; moving 1 or 2 cm/day		May persist for months
<i>Bunostomum phlebotomum</i>	Cattle	Filariform?	Papules; migration for few mm		2 weeks
<i>Gnathostoma spinigerum</i>	Cat, dog, pig	Filariform?	Deep burrows, band-like infiltrates, at times furunculoid	Gnathostomiasis; Yangtze oedema, migrating oedema	May be active for years
<i>Enterobius vermicularis</i>	Human	Rhabditiform	Vesiculopustular eruption about perianal region	Pinworm infection; oxyuriasis	Months
<i>Strongyloides stercoralis</i>	Human, cat, dog	Filariform	Papules, redness and itching, or urticarial band extending as much as 10 cm/day; originates in perianal area	Strongyloidiasis; strongyloides ground itch; larva currens	Weeks; recurrent for years
<i>Rhabditis strongyloides</i>	Dog	Rhabditiform	Dome-like, pruritic papules with follicular arrangement	Dermatitis rhabditidosa	2-5-3 months

* Adapted from Harman (1969) and Smith *et al.* (1976).

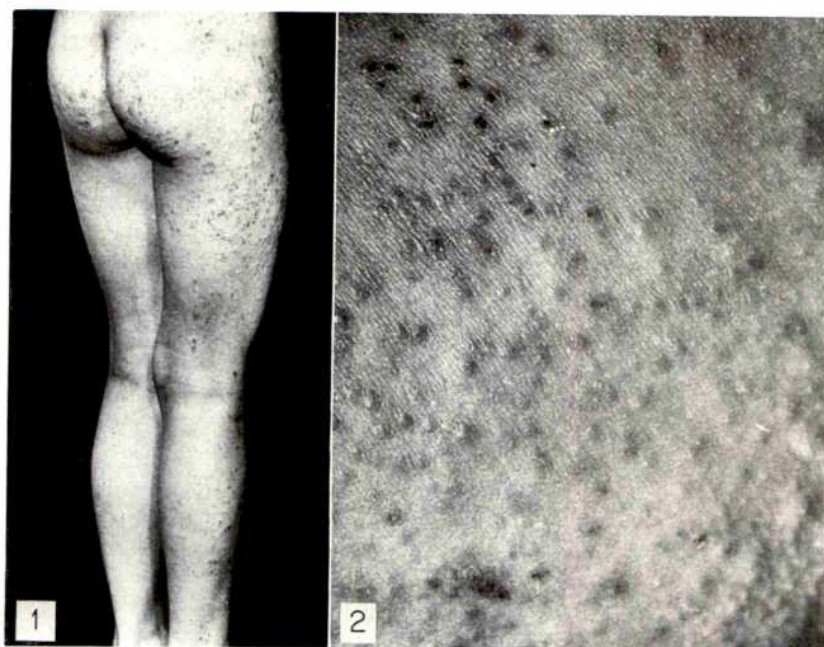


FIGURE 1. Dermatitis rhabditidosa in an 11-year-old girl.

FIGURE 2. Skin lesions: domelike papules with follicular arrangement.

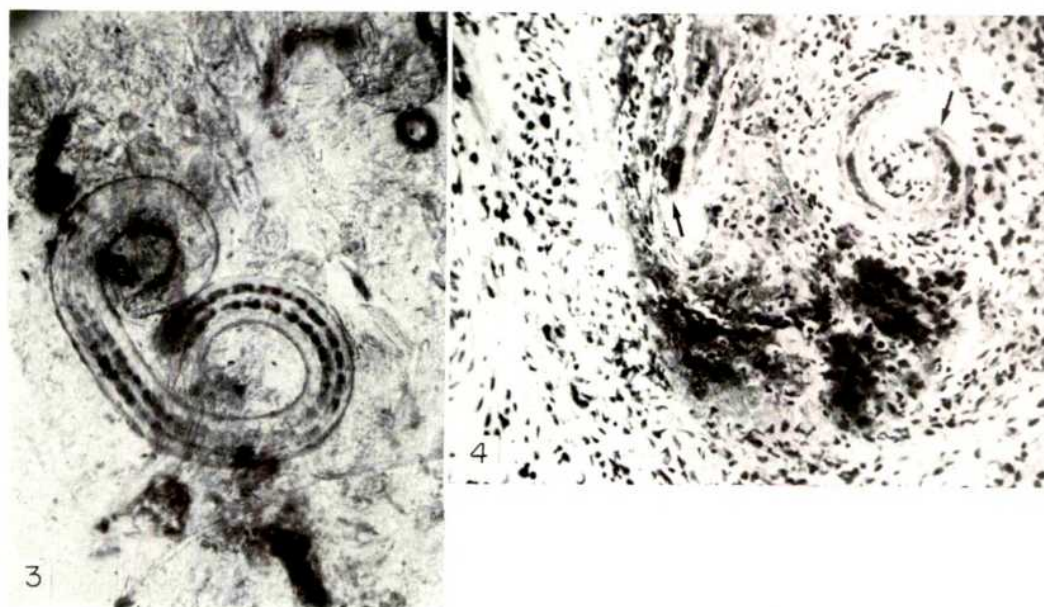


FIGURE 3. A coiled larva of *Rhabditis strongyloides* isolated from the child's skin ($\times 450$).

FIGURE 4. A few fragments of larvae in the necrotic hair follicle surrounded by a mononuclear infiltration, eosinophils and a homogeneous area. (Biopsy specimen of the child's skin.) (H & E, $\times 450$).

papules of skin colour or light brown, domelike in form, with follicular arrangement, 2–4 mm in diameter, and numerous scratch marks (Figs 1 and 2).

In skin scrapings live rhabditiform larvae were found, about 0.7 mm in length and 0.02–0.03 mm in diameter (Fig. 3), which were confirmed upon every examination for a period of 6 weeks, despite repeated treatment with crothamiton ointment, Rivanol-salicylic ointment, and baths. In this period biopsy specimens from the skin papules were taken twice for histological study.

After two and a half months from the onset of the skin lesions, the itching decreased and the papules began to subside. In the skin scrapings dead larvae were found. During the next 3 weeks papules and itching disappeared completely leaving patchy scars and hyperpigmentation.

Laboratory studies showed a blood eosinophilia of 10%. Examination of faeces for parasites was negative 8 times though on one occasion in Hall's method (NIH) (Frankel *et al.*, 1963) around the atra of the anus a live *Oxyuris vermicularis* was found. In the duodenal aspirate, saliva and vaginal smears no parasites were found. Urine was sterile on culture. The ESR was 9 mm/h. Serum proteins: albumin 39%; globulins: alpha₁ 9.1%, alpha₂ 16.5%, beta 15.8%, gamma 19.6%. Serum immunoglobulins: IgM 190 mg%; IgG 1700 mg%; IgA 155 mg%. Liver function tests and serum electrolytes were normal.

Histological examination of the skin biopsy revealed the frontal part of the larva's cuticle lying in a fissure in the stratum corneum. A few fragments of larvae were found in necrotic hair follicles in the infiltrate, which chiefly consisted of mononuclear cells and eosinophils. The infiltrate extended

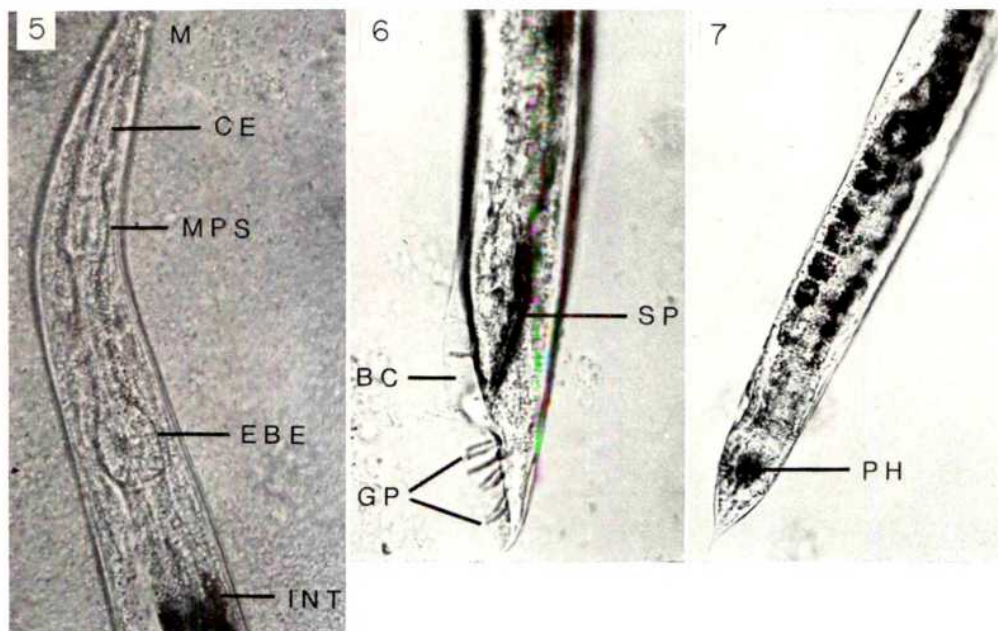


FIGURE 5. *Rhabditis strongyloides*. Frontal part of the nematode. M—mouth, CE—corpus of oesophagus; MPS—median pseudobulb; EBE—end bulb of oesophagus; INT—intestine. Culture ($\times 450$).

FIGURE 6. *Rhabditis strongyloides*. Caudal part of the male nematode. SP—spicula; BC—bursa copulatrix; GP—genital papillae. Culture ($\times 450$).

FIGURE 7. *Rhabditis strongyloides*. Caudal part of the female nematode. PH—phasmidium. Culture ($\times 450$).

down the sheath of the follicle and penetrated it. There were small, similar infiltrates around capillaries and venules of the upper dermis (Fig. 4).

To identify the parasite, cultures were set up, from larvae found in the child's skin, on 2% agar with one piece of fresh beef in the plate. After 7 days the larvae matured, started to multiply and showed typical features of *Rhabditis strongyloides* (Figs 5-7).

In the search for the source of the child's infestation with the larvae, the parents were examined but no larvae were found in their skin, faeces or saliva. Since the dog had been buried in a plastic bag for 7 weeks, during a period of sub-zero temperatures, a partial autopsy was undertaken. In the dog, weighing about 6 kg, extensive thinning of the long hairs was observed around the abdomen and left foreleg. No parasites were found in the intestines either macro- or microscopically. Nor were there any in the oral cavity or nasal passages. In hairs taken from the thinning sites, as well as from the anal area and thick tail, numerous dead and live larvae (similar to those from the child's skin) and mature nematodes—*Rhabditis strongyloides*—were found, which in meat culture multiplied within 5 days.

DISCUSSION

The infestation of the child might have been the result of frequent hugging of the infected dog and the transfer by the hands of larvae onto the skin, or both the child and the animal might have been infested from the usual environmental sources. We were able to cultivate directly mature forms from the parasitic larvae from the child's skin. The post mortem examination of the dog (isolated by plastic which prevented the invasion of larvae from the ground) confirmed the thinning of the hair and revealed larvae and mature forms of *Rhabditis strongyloides*. The long period in which the larvae survived in the skin of the child was probably due to the appropriate pH of the skin and the concentration of mineral salts in the sweat. The studies of Soroczán & Krauze (1973) showed that the optimal pH for the development of *Rhabditis strongyloides* is with weak and very weak acid buffers of pH 5.0-6.5. All the developmental forms of *Rhabditis str.* tolerated well the effect of moderately high concentrations of mineral salts, but the type of salt played a substantial role. Some changes in pH destroyed the larvae and mature nematodes. These data are of importance when selecting drugs against this parasite.

On the basis of cytological and histological studies one can assume that the *Rhabditis str.* larvae can live in man in the upper layers of the epidermis and penetrate deeply into the hair follicle along a hair. These parasites cause moderate inflammation around the blood vessels in the upper layers of the skin and solid infiltration of the hair follicles with complete destruction of the follicles and papillae. In consequence, the hairs (lanugo) become loosened and fall out leaving small scars. Besides, dead or live worms presumably release antigenic or toxic substances into the skin. It is not known whether the pruritus is related mainly to trauma caused by migratory larvae, to metabolic products of living parasites, or to the release of antigens upon the death of the worms.

In the differential diagnosis we considered the possibility of an autosuperinfection by *Strongyloides stercoralis*, but we failed to find the latter either in the saliva, duodenal contents or faeces of the child and her parents. Moreover, the invasive larvae of *Strongyloides stercoralis* are filariform with a cylindrical oesophagus and not rhabditiform as in our patient.

More probable seemed the invasion by rhabditiform larvae of the pinworm (*Oxyuris vermicularis*) into the skin of the child, but on the basis of the tests (8 times the faeces examination was negative, and in the anal area only 1 was found) we identified a very small infection which could not possibly have caused the skin lesions.

The rhabditiform larvae found on the child's skin and the acquisition of a culture of mature *Rhabditis strongyloides* are good evidence of a parasitic skin infestation in man.

ACKNOWLEDGMENT

The author is indebted to Dr Wiesław Soroczan from the Department of Biology and Parasitology of the Biological-Morphological Institute of the Medical Academy in Lublin, Poland, for his help in identifying the parasites.

REFERENCES

- CHENG, T.C. (1964) *The Biology of Animal Parasites*, p. 415. W.B. Saunders Company, Philadelphia and London.
- CHITWOOD, B.G. (1932) The association of *Rhabditis strongyloides* with dermatitis in dogs. *North American Veterinarian*, **13**.
- FRANKEL, S., REITMAN, S. & SONNENWIRTH, A.C. (1963) *Gradwohl's Clinical Laboratory Methods and Diagnosis*, pp. 950-951. C.V. Mosby, Saint Louis.
- HARMAN, R.R.M. (1969) Parasitic worms and protozoa. In: *Textbook of Dermatology* (Ed. by A. Rook, D.S. Wilkinson and F.J.G. Ebling), p. 944. Blackwell Scientific Publications, Oxford.
- HAYES, J.E. (1953) Nematode dermatitis in the dog caused by *Rhabditis strongyloides*. *North American Veterinarian*, **34**, 8.
- HELLEBRAND, L. (1924) Niezwykły przypadek zapalenia skóry u psa, wywołany przez larwy oblenców. *Przegląd Weterynaryjny*, **37**, 6.
- MULLER, G.H. & KIRK, R.W. (1969) *Small Animal Dermatology*, pp. 137 and 357. W.B. Saunders, Philadelphia.
- RHODE, E.A., JASPER, D.E., BAKER, N.F. & DOUGLAS, J.R. (1953) The occurrence of *Rhabditis dermatitis* in cattle. *North American Veterinarian*, **34**, 9.
- ŚLUSARSKI, W., MARKIEWICZ, K. & STEFAŃSKI, W. (1956) Sur un cas de dermatite vermineuse chez le chien provoquée par l'invasion des larves *Rhabditis strongyloides* Schneider 1866. *Acta Parasitologica Polonica*, **4**, 163.
- SMITH, J.D., GOETTE, D.K. & ODOM, R.B. (1976) Larva currens; cutaneous strongyloidiasis. *Archives of Dermatology*, **112**, 1161.
- SOROCZAN, W. & KRAUZE, M. (1973) The effect of pH and mineral salt concentration of the medium under laboratory conditions on the survival and growth of the round worm *Rhabditis strongyloides* Schneider, 1886. *Annales Universitatis Mariae Curie-Skłodowska Lublin—Polonia, Sectio DD*, **28**, 207.
- STEFANŃSKI, W. (1959) *Glista ludzka i inne nicienie*. P.W.N. Warszawa.
- STEFANŃSKI, W. & ŻARNOWSKI, E. (1971) *Rozpoznawanie inwazji pasożytniczych u zwierząt*, pp. 49, 85, 280. PWRL Warszawa.
- THORNE, G. (1961) *Principles of Nematology*, p. 454. McGraw-Hill Publications in the Agricultural Sciences.

Brief Communication

Iatrogenic alterations of the biochemical properties of human skin

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Pharmacological alterations of the connective tissue matrix can be evaluated by the modifications they produce in the mechanical properties of the dermis (Burton & Shuster, 1974). Using our technique, the rheological properties of skin are evaluated *in situ* by the ability of the forearm skin to withstand vertical forces of extension provided by a specially designed stretching device (Pierard, 1973, 1976; Pierard & Lapière, 1976). The vertical extensibility (E) and the ability of the skin surface to regain its initial shape and position after deformation (Biological elasticity, BE) are recorded.

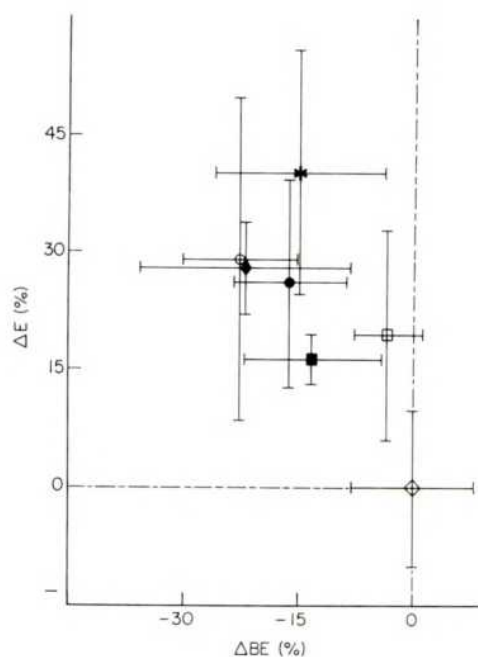


Fig. 1.

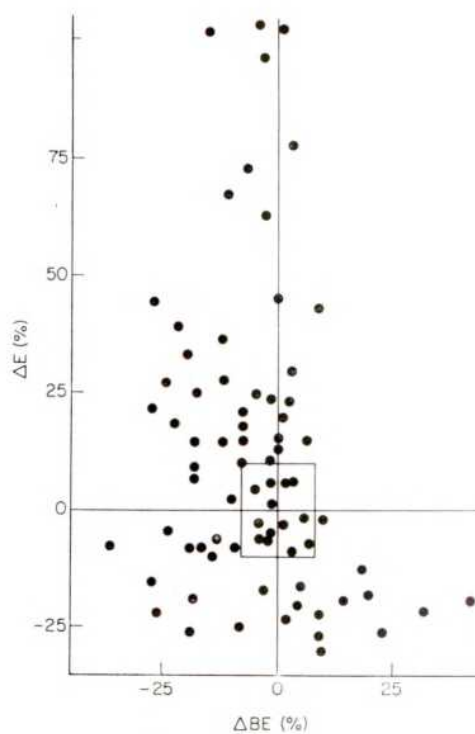


Fig. 2.

FIGURE 1. Corticosteroid-induced atrophy (increased E and decreased BE); induced tension within the skin = 1 Newton.

(◇) Normal value and standard deviation; (□) hydrocortisone 0.5% (3 patients); (■) hydrocortisone butyrate 0.1% (Locoid) (15 patients); (●) clobetasone butyrate 0.05% (Molivate) (6 patients); (◊) fluocinonide 0.05% (Topsione) (4 patients); (○) fluocinolone acetonide 0.025% (Synalar) (7 patients); (*) clobetasol propionate 0.05% (Dermovate) (12 patients).

FIGURE 2. Photochemotherapy-induced atrophy (increased E and decreased BE) and sclerosis (decreased E and increased BE); induced tension within the skin = 1 Newton. Variations within the normal population correspond to the inserted central border.

Two types of iatrogenic alterations of the connective tissue function have been evaluated: the corticosteroid and the photochemotherapy-induced remodellings.

Under topical corticotherapy, the extensibility of skin for low stress is increased and the biological elasticity is reduced. For higher stress the biological elasticity overcomes the normal values (Pierard, 1976; Pierard *et al.*, 1976). Preliminary results, comparing in normal adults (25–45 years of age) several topical corticosteroids after 2 weeks of twice daily non-occlusive application in their conventional ointment base provided by the pharmaceutical industry, are presented in Fig. 1. The potency to induce atrophy varies significantly.

Photochemotherapy administered by the PUVA system (psoralen and UVA) according the ECCT (European Cooperative Clinical Trial) schedule (Wolff *et al.*, 1977) can induce either a photoatrophy or a photosclerosis (Pierard, de la Brassinne & Lapière, 1977). During a one year follow up, most patients evolve towards atrophy (increased E and decreased BE) while others present a decreased E and BE related to a long standing oedema evolving towards sclerosis. It is characterized by a decreased E and an increased BE (Fig. 2). The high risk conditions predisposing the patients to one or the other of these remodellings of the superficial dermis are dependent on dose and rhythm of UVA irradiation and on the photosensitivity of the skin rated according to Wolff *et al.* (1977).

Measurements of the rheological properties of skin can be helpful in monitoring therapy and the side effects of drugs. It is a fast and non-traumatizing procedure providing more complete information than the measurement of skin thickness (Kirby & Munro, 1976).

REFERENCES

- BURTON, J.L. & SHUSTER, S. (1973) A rapid increase in skin extensibility due to prednisolone. *British Journal of Dermatology*, **89**, 491.
- KIRBY, J.D. & MUNRO, D.D. (1976) Steroid-induced atrophy in an animal and human model. *British Journal of Dermatology*, **94**, suppl. **12**, 111.
- PIERARD, G.E. (1973) Skinfold thickness, skin surface deformability and connective tissue architecture. *3rd Meeting of the European Society for Dermatological Research*, Abstract No. 28, Amsterdam.
- PIERARD, G.E. & LAPIÈRE, CH.M. (1976) Skin in dermatosparaxis. Dermal microarchitecture and biomechanical properties. *Journal of Investigative Dermatology*, **66**, 2.
- PIERARD, G.E. (1976) Microanatomie et propriétés fonctionnelles du tissu conjonctif de la peau. Influence de la corticothérapie locale et systémique. In: *La Corticothérapie Locale* (Ed by Ch.M.Lapière and G.E.Pierard), p. 135. Brocades Belga Publ. Div. Bruxelles.
- PIERARD, G.E., HERMANN, J.F., LACHAPPELLE, J.M. & LAPIÈRE, CH.M. (1976) Microanatomical and functional aspects of corticosteroid-induced atrophy of skin. *Archives Internationales de Physiologie et de Biochimie*, **84**, suppl. **3**, 37.
- PIERARD, G.E., DE LA BRASSINNE, M. & LAPIÈRE, CH.M. (1977) Effects of long term photochemotherapy on the dermis. *Journal of Investigative Dermatology*, **68**, 249.
- WOLFF, K., GSCHNAIT, F., HONIGSMANN, H., KONRAD, K., PARRISH, J.A. & FITZPATRICK, T. (1977) Phototesting and dosimetry for photochemotherapy. *British Journal of Dermatology*, **96**, 1.

Obituaries

PROFESSOR J. PIÑOL AGUADÉ

Joaquín Piñol Aguadé, Professor of Dermatology in Barcelona, died aged 59 on 17 August 1977.

Soon after he qualified at Barcelona in 1942, he studied dermatology under Professors Jaime Peyri and Xavier Vilanova at Barcelona, and Professors Gay Prieto, Sainz de Aja and Gomez Orbaneja at Madrid. On the death of Professor Vilanova in 1967, he succeeded to the Chair of Dermatology and Venereology in Barcelona.

Perhaps his best known original contribution to dermatology was in 1956 when, with Vilanova, he first described Hypodermite Nodulaire Subaiguë Migratrice (or Subacute Nodular Migratory Panniculitis). He more recently became interested in the porphyrias and in photobiology and in 1969 first described what is now recognized as a new type of porphyria, 'Hepatoerythrocytic Porphyria'. From this developed a happy collaboration with U.K. colleagues in the Institute of Dermatology, London, and the Porphyrin Metabolic Unit, Cardiff.

Piñol Aguadé was of a genial and generous, yet modest, personality; he was also a man of enormous energy and an excellent organizer and administrator, as shown in the 4 International Dermatology Meetings he staged in Barcelona between 1969 and 1975. He was also a member of numerous learned Societies—Argentinian, Brazilian, British, French, Italian, Portuguese, Spanish, etc. As well as this, he was a great writer, responsible for over 400 papers, many in foreign languages in many journals and had also written a number of books and monographs.

Professor Piñol was proud to be a Catalan and native of the Province of Gerona. He numbered lawyers and scholars in his family. His energy and scholarship are typical of the Catalan in his dedication to a very heavy daily schedule of work, clinical, laboratory, teaching and administrative.

We extend our sympathy to his wife, son and daughter.

I.A. MAGNUS



DR ELIZABETH HUNT

Dr Elizabeth Hunt, formerly dermatologist to the South London Hospital for Women, and the New Sussex Hospital for Women, Brighton, died on 23 July 1977 at the age of 101.

Elizabeth Adams was born at Omagh, County Tyrone, in 1876 and graduated B.A. at the Royal University of Ireland. In 1906 she married Dr T.H. Hunt who was in practise in Halifax. In 1918 she was widowed and then decided on medicine as a career. After a year at Leeds she moved to Liverpool University where she qualified in 1922, proceeding M.D. in 1924. Post-graduate work in Vienna, Paris and London led to a research scholarship at the Liverpool Royal Infirmary in the Skin Department under the late Dr Robert Mackenna. Later she became temporary honorary dermatologist at the Royal Infirmary, Liverpool, and at the Radium Institute and Hospital for skin and Cancer in that city.

In 1930 Dr Hunt moved to London and became dermatologist at the South London Hospital for Women and the New Sussex Hospital for Women, Brighton. When Dr J.H. Twiston Davies volunteered for army service she took charge of the skin department at the Royal Sussex County Hospital, Brighton, and undertook an enormous work load during the War years. Her book, *Diseases Affecting The Vulva*, first published in 1940 ran into four editions; it was based on her own personal observations of a study of over a thousand cases of vulval affection, and was illustrated with excellent colour photographs which would shame many current publications. It remains a work of reference for dermatologists and gynaecologists.

Elizabeth Hunt is remembered as a small energetic woman with a clear incisive mind which served her well on medical staff committee meetings, and in the larger arena of the Medical Women's Federation and on the Soroptimists International Association when she became President of the European Federation in 1930. She was meticulous in all that she undertook.

Three of her nephews were doctors and achieved eminence in their chosen fields of medicine. In her centennial year she was elected an honorary member of the British Association of Dermatologists.

PATRICK HALL-SMITH

British Journal of Dermatology (1978) **98**, 117.

Book reviews

The Physiology and Pathophysiology of the Skin. Volume 4, The Hair Follicle. Edited by A. JARRETT (1977) London; Academic Press. Pp. xx, 304, xxi, illustrated. Price £18.50.

This is the fourth in the series of comprehensive surveys of the physiology and pathophysiology of the skin, edited by Dr Jarrett of the Department of Dermatology, University College Hospital Medical School, London. In this volume he has been assisted by two zoologists, Drs Johnson and Spearman, both well known for their research on various aspects of the pathophysiology of the skin. Dr Elizabeth Johnson has published numerous important papers on hair in several different species of mammal; some of her earlier work was carried out in collaboration with Dr Ebling in Sheffield, but for some years she has continued her investigations with other colleagues at the University of Reading. Dr Spearman's name is known to dermatologists mainly for his valuable studies of keratinization. Dr Jarrett has therefore chosen a small but impressive team and it is no surprise that the product of their labours is a scholarly and lucid book which can be most strongly recommended to anyone who is interested in the intricate biology of hair growth, whether as a laboratory worker, a clinician, or a scientifically inclined sheep farmer.

Eight of the nine chapters cover systematically all aspects of the biology of the hair follicle and skillfully use findings based on detailed observations in other mammals to throw light on phenomena which have been less carefully investigated in man. The coverage of the world literature has been wide, and the references are conveniently grouped at the foot of the page on which they are cited. The references are in general well selected, but the authors often cite secondary rather than primary sources. This may be a deliberate policy, because secondary sources are often more readily accessible than the original research articles, but it means that some investigators are not given the credit they deserve.

The last chapter, on abnormal hair growth in man, is perhaps the least successful in the book. This weakness is not the fault of the authors, but is the almost inevitable consequence of attempting to compress a very large subject into less than twenty pages of text. Compression involves both omission and simplification and necessitates generalizations which tend to be misleading when applied to clinical syndromes of almost bewildering heterogeneity.

The book is well produced and effectively illustrated. It is certainly the best reference book available on the hair follicle, but so to label it without some qualification does it less than justice. An important work of reference it undoubtedly is, but it should not be immediately consigned to the shelf, and consulted only when the occasion arises. It provides an extremely readable account of a subject of great practical importance to dermatologists, and does so in the context of general biological principles and can be read with enjoyment as well as with profit.

ARTHUR ROOK

Dermatotoxicology and Pharmacology: Toxicology Vol. 4. Edited by FRANCIS N. MARZULLI and HOWARD I. MAIBACH (1977) Chichester: John Wiley & Sons Ltd. Pp. 467. Price £26.00

This book represents one volume of a series entitled *Advances in Modern Toxicology* and embodies a number of review articles on various aspects of a new discipline known, rather ponderously, as dermatotoxicology. The impetus for the foundation of the sub speciality came, not as one might have expected from within the ranks of dermatologists, but from demands and requirements of recent U.S. government regulations on consumer safety.

In their preface the editors indicate that some difficulties had been experienced in meeting publication deadlines and one chapter in particular gives the distinct impression that the editorial hand had been too lightly laid upon it. Unfortunately this contribution comes first in the book and should not be allowed to discourage the type of reader who approaches the work as a novel and reads directly from cover to cover.

From their content it would appear that these review articles are addressed to knowledgeable scientific readers to provide basic, up-to-date facts on subjects with which they may be unfamiliar.

Whatever their purpose they provide enjoyable spare time reading for the slightly out-of-date physician on a variety of important, basic subjects relating to skin function, irritation, allergy and cutaneous microbiology. There is even a highly mathematical chapter propounding a skin permeability theory which I have no doubt will be incomprehensible to most readers of this journal.

An estimated 75% of the book, however, is of major interest to the practising dermatologist. Articles by Ebling & Hjorth on their own subjects are, as always, well written and informative. It is difficult to try and choose the best from a first class international selection of authors.

I would commend as particularly noteworthy a chapter by Polak on Immunological aspects of Contact Sensitivity which should be required reading for us all and perhaps justifies the purchase price on its own merits.

Obviously a proportion of the articles are very directly related to toxicological testing methods in the laboratory environment and will have less interest to the office dermatologist. Whether Dermatotoxicology stands or falls as a speciality depends very much on the establishment of reproducible tests of irritancy, sensitization and carcinogenic potentials of the vast quantities of new chemicals to which we seem destined to be exposed throughout our lifetimes. On the evidence presented by Drs Marzulli and Maibach the foundations of dermatotoxicology stand firm indeed and they deserve every congratulation for their efforts.

F.A.IVE

Lymphedema. Supplement to Lymphology. 1977. Edited by LEO CLODIUS (1977) Stuttgart; Georg Thieme. Pp. viii, 192, 158 figs, 35 tables.

As the editor of this book, Dr Clodius, says in his preface, research into the form and function of lymphatics lags behind the study of the circulation of other body fluids. In addition to this there is a paucity of literature on lymphatics available in the English language. This book, which is a collection of papers arising out of the Fourth International Congress of Lymphology, is therefore particularly welcome.

It deals with the physiology and pathophysiology of lymph flow, with the production and treatment of experimental lymphoedema and with the treatment of clinical lymphoedema mainly of the limbs, and is chiefly surgical in its approach.

From the dermatologist's angle there is an interesting article on filarial elephantiasis, and the early chapters on physiology and pathology of lymph flow and the ultrastructure of the lymphatic system in lymphoedema are stimulating, especially the work on using Venalot (Coumarin and Troxerutin) in the management of chronic lymphoedema. This increases ^{125}I albumin clearance and is thought to act by increasing protein catabolism in the interstitial fluid. It is disappointing that only 8 of the 190 pages are devoted to lymphoedema of the face and this chapter too is purely surgical, no mention at all being made of lymphoedema of lips or ears.

The book suffers from two of the defects of multiple authorship, namely repetition and patchiness in the quality of the chapters. One of the authors enthuses over particular surgical techniques while giving no documented evidence to justify his enthusiasm. Perhaps a little of the editorial restraint which the editor stresses that he has not used would have been helpful here. The English too is sometimes rather hard to follow, probably because of translation problems. Despite these criticisms, however, it is of real value to have a collection of papers by many of the foremost workers in the lymphatic field combined into one slim English volume. The presentation, photographs, lymphangiograms and diagrams are excellent and there are extensive references to international lymphatic literature. I would suspect that the book is too technical and too surgically orientated to demand a place on the clinical dermatologist's book shelves, but it will be a useful reference book for anyone interested in the lymphatic field.

JULIA P.ELLIS

Comment

TYROSINE-INDUCED SKIN DISEASE

Tyrosinaemia is associated with a distinctive oculo-cutaneous syndrome in both man and rodents. The characteristics of this newly recognized syndrome and its pathophysiology are the subjects of this review.

Clinical

Seven patients have been reported who clinically have a syndrome of palmar and plantar hyperkeratoses, corneal ulceration, tyrosinaemia, tyrosinuria and phenolicaciduria (Hill & Zaleski, 1971; Holston *et al.*, 1971; Kennaway & Buist, 1971; Burns, 1972; Goldsmith *et al.*, 1973; Zaleski, Hill & Kushniruk, 1973; Billson & Danks, 1975; Sandberg, 1975; Goldsmith & Reed, 1976). The syndrome is known as Tyrosinaemia II. Both males and females are affected and patients of Italian, Norwegian, Anglo-Saxon and American Black racial background have been reported. Three of the patients have come from consanguineous marriages, favouring an autosomal recessive mode of inheritance.

Eye symptoms usually begin during the first few months of life and consist of lacrimation, redness and photophobia. Eye signs include corneal clouding, dendritic ulcers, and in one patient a persistent conjunctival plaque (Bienfang, Kuwabara & Pueschel, 1976). Bacterial and herpes cultures are negative and topical therapy is ineffective.

Skin lesions begin in the first few months of life, usually after the eye lesions, and are limited to the palms and soles. The lesions are initially erosions which then rapidly become crusted, hyperkeratotic and painful. The lesions may be linear and are most prominent on the distal phalanges, thenar eminences and hypothenar eminences. They are unresponsive to the usual dermatological therapies.

Some of the patients have had moderate to severe mental retardation and one of the patients had multiple congenital anomalies (Burns, 1972).

The parents of patients are normal and have essentially normal tyrosine levels (Goldsmith, 1977).

Metabolic studies

By definition, all of the patients have had tyrosinaemia with blood tyrosine levels between 30 and 60 mg/dl (normal less than 2.5 mg/dl), tyrosinuria, and increases in the urinary tyrosine metabolites p-hydroxyphenylpyruvate, p-hydroxyphenyllactate, p-hydroxyphenylacetate, N-acetyltyrosine, and p-tyramine. Amino acids other than tyrosine are characteristically normal; renal and hepatic function tests are also normal; these characteristics distinguish this disease from other disorders of tyrosine metabolism (*vide infra*).

Tyrosinaemia in tyrosinaemia II is caused by a deficiency of hepatic tyrosine aminotransferase (L-tyrosine-2-oxoglutarate aminotransferase, E.C.2.6.1.5) as determined by a liver biopsy study in one patient with the disease (Fellman *et al.*, 1969). No further characterization of the mutant enzyme has been performed.

The presence of increased levels of tyrosine metabolites in the absence of tyrosine aminotransferase is explained by mitochondrial aspartate aminotransferase catabolizing tyrosine (Fellman, Fujita & Roth, 1972).

Therapy

The tyrosinaemia, the eye lesions and the skin lesions respond to a low-tyrosine low-phenylalanine

diet (Mead Johnson 3200 AB). Patients have been maintained on this diet supplemented with some normal food for up to 2 years with normal growth and development. If the diet is stopped the disease recurs. Patients have not responded to cortisone, ascorbic acid, pyridoxine or folic acid.

Pathology

Routine biopsy of the skin lesions was not diagnostic and showed acanthosis and hyperkeratosis. Electronmicroscopy of one of the palmar lesions showed 2–3 μm lipid-like granules and myelin figures suggestive of lysosomal activation (Goldsmith *et al.*, 1973). Biopsy of a persistent conjunctival plaque in one patient showed Alcian-blue positive inclusions, needle-like inclusions, and whorled membranous substances (Bienfang *et al.*, 1976).

Differential diagnosis

The *Richner-Hanhart syndrome* (RHS) consists of keratosis palmoplantaris, persistent dendritic lesions of the cornea, frequently profound mental retardation and autosomal recessive inheritance. Franceschetti, Schnyder & Felgenhauer (1971) reviewed fourteen cases of this classical syndrome. Several of the patients with tyrosinaemia II have been diagnosed as having the RHS and it is possible that most of the patients with classical RHS have had tyrosinaemia as the basis of their disease. Every patient with the diagnosis of RHS deserves the appropriate tests for tyrosinaemia.

Other metabolic disorders with abnormalities in tyrosine metabolism are reviewed elsewhere (Scriver & Rosenberg, 1973; Buist, Kennaway & Fellman, 1974). Tyrosinaemia (tyrosinosis; tyrosinaemia, type I) is associated with liver and renal disease and has abnormalities in liver and renal function tests and abnormalities in the blood and urine levels of several amino acids. Neonatal tyrosinaemia is responsive to vitamin C supplementation and a moderate reduction in dietary protein; it does not have eye or skin lesions and is a self limited disorder. Other and even rarer disorders of tyrosine metabolism, also without skin or eye lesions, are reviewed elsewhere (Goldsmith, 1977).

Animal model for tyrosinaemia

Schweizer (1947) fed rats a low protein diet supplemented with large amounts of L-tyrosine and produced a syndrome with keratitis, conjunctivitis, alopecia, cheilitis, and inflammatory changes on the paws. The skin and eye lesions occur within days. The tyrosinaemic rat has proved to be a useful experimental model in several laboratories including ours. Young rats and male rats are more severely affected than older or female rats. The syndrome is ameliorated or prevented by increases in the dietary protein, L-threonine supplementation (Alam *et al.*, 1966), thiouracil, glucagon (Ip & Harper, 1973), phenobarbitone (Selye, 1971), glucocorticoids and catatoxic steroids devoid of glucocorticoid activity (Selye, 1971). These agents probably act by increasing the levels of hepatic tyrosine aminotransferase thus reducing tyrosine below toxic levels. Since rats fed p-hydroxyphenylpyruvate do not develop the syndrome, tyrosine, and not its p-hydroxylated metabolites, are implicated in causing the syndrome.

Eye lesions in tyrosinaemic rats

The induction of eye lesions in the rats has been extensively studied (Rich, Beard & Burns, 1973; Beard *et al.*, 1974; Gipson, Burns & Wolfe-Lande, 1975). Long, slender birefringent crystals were limited to the areas of corneal damage. They passed through cell and nuclear membranes and were sometimes membrane bound. Autophagic vacuoles and multivesicular bodies were seen electronmicroscopically (Gipson *et al.*, 1975). A dense polymorphonuclear infiltrate of the corneal stroma and epithelium was a characteristic feature of the lesion.

Skin lesions in tyrosinaemic rats

In my laboratory (unpublished studies) we found lesions limited to the bottom of the paws. Erosions, crusts and hyperkeratosis were seen, as was subungual involvement leading to the separation of nails. Focal areas of dyskeratosis were followed by dense infiltrates of polymorphonuclear leukocytes which later formed subcorneal collections.

Lesions were strictly limited to volar and subungual epidermis. Biopsy of tail or back epidermis, even after stimulation of the epidermis by hair plucking, showed no evidence of epidermal change or inflammation. Comparable levels of tyrosine and its metabolites were found in volar and tail skin by chemical analysis.

Although the skin lesions in the rat contained more inflammatory cells than has been seen in biopsies from patients with tyrosinaemia, the similarity of localization of lesions to the cornea and volar epidermis in both the human disease and the animal model is remarkable and no doubt this model will be exploited further.

Effect of tyrosine crystals on membranes

It is hypothesized that tyrosine crystals, and not tyrosine in solution, are causally related to the production of the epithelial lesions. Tyrosine itself is not a potent chemotactic agent and even tyrosine crystals when placed in the skin or peritoneal cavity did not produce inflammatory responses. Therefore, we suggest that when tyrosine *crystallizes* within cells it initiates the inflammatory cascade. Data in support of this hypothesis are that tyrosine crystals can specifically labilize lysosomes (Goldsmith, 1975) and can specifically haemolyse erythrocyte membranes (Goldsmith, 1976). These effects are related to the active phenolic groups present on the surface of the crystals. Plasma tyrosine concentrations in tyrosinaemia II and in tyrosinaemic rats approach chemical saturation; normally, epidermis has higher levels of free tyrosine than does plasma (Tabachnick & LaBadie, 1970). Once within the epidermis, it is presumed that, due to pH changes, hydration changes, dissociation from carrier proteins, etc. the tyrosine crystallizes. Tyrosine crystals when they interact with lysosomes or other membrane associated structures may rupture the organelle, releasing proteases and other chemotactic factors initiating the inflammatory cascade. In C5 deficient mice fed a high tyrosine diet, eye and skin lesions occurred (unpublished observation) suggesting that the complete complement cascade was not necessary to produce the lesions.

Crystalline urates (Weissmann & Rita, 1972), silicates (Nash, Allison & Harington, 1966), calcium pyrophosphate dihydrate crystals (Kozin & McCarty, 1976), calcium hydroxyapatite (Dieppe *et al.*, 1976), and protoporphyrins (Bloomer *et al.*, 1975; Gschnait *et al.*, 1975) are presumed to cause tissue damage through similar mechanisms by the activation of lysosomes. The ability of a crystal to initiate acute inflammation is related to its surface chemistry (Mandel, 1976); a variety of crystalline materials, even those which are deposited within lysosomes (e.g. cystine in cystinosis), do not cause inflammation.

We expect that the recognition of the molecular basis and the treatment of the Richner-Hanhart syndrome will lead to recognition of more cases of this previously untreatable disease. The renewed interest in the animal model for tyrosinaemia should allow more detailed studies of inflammatory mechanisms in the skin and may answer questions concerning the localization of certain diseases of volar skin. Furthermore, the interaction of tyrosine crystals and membranes is a novel system for investigating some properties of membranes.

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REFERENCES

- ALAM, S.Q., BECKER, R.V., STUCKI, W.P., ROGERS, Q.R. & HARPER, A.E. (1966) Effect of threonine on the toxicity of excess tyrosine and cataract formation in the rat. *Journal of Nutrition*, **89**, 91.
- BEARD, M.E., BURNS, R.P., RICH, L.F. & SQUIRES, E. (1974) Histopathology of keratopathy in the tyrosine-fed rat. *Investigative Ophthalmology*, **13**, 1037.
- BIENFANG, D.C., KUWABARA, T. & PUESCHEL, S.M. (1976) The Richner-Hanhart syndrome. Report of a case with associated tyrosinemia. *Archives of Ophthalmology*, **94**, 1133.
- BILLSON, F.A. & DANKS, D.M. (1975) Corneal and skin changes in tyrosinaemia. *Australian Journal of Ophthalmology*, **3**, 112.
- BLOOMER, J.R., PHILLIPS, M.J., DAVIDSON, D.L. & KLATSKIN, G. (1975) Hepatic disease in erythropoietic protoporphyria. *American Journal of Medicine*, **58**, 869.
- BUIST, N.R.M., KENNAWAY, N.G. & FELLMAN, J.H. (1974) *Disorders of tyrosine metabolism in heritable disorders of amino acid metabolism*, (ed. by W. L. Nyhan), p. 160. J. Wiley & Sons, New York.
- BURNS, R.P. (1972) Soluble tyrosine aminotransferase deficiency: an unusual cause of corneal ulcers. *American Journal of Ophthalmology*, **73**, 400.
- DIEPPE, P.A., CROCKER, P., HUSKISSON, E.C. & WILLOUGHBY, D.A. (1976) Apatite deposition disease—a new arthropathy. *Lancet*, **i**, 266.
- FELLMAN, J.H., FUJITA, T.S. & ROTH, E.S. (1972) Assay, properties and tissue distribution of p-hydroxyphenylpyruvate hydroxylase. *Biochimica et Biophysica Acta*, **284**, 90.
- FELLMAN, J.H., VANBELLINGHEN, P.J., JONES, R.T. & KOLER, R.D. (1969) Soluble and mitochondrial forms of tyrosine aminotransferase. Relationship to human tyrosinemia. *Biochemistry*, **8**, 615.
- FRANCESCHETTI, A.TH., SCHNYDER, U.W. & FELGENHAUER, W.R. (1971) Die Cornea beim Richner-Hanhart Syndrom. *Bericht; Deutsche Ophthalmologische Gesellschaft*, **LXXX**, 109.
- GIPSON, I.K., BURNS, R.P. & WOLFE-LANDE, J.D. (1975) Crystals in corneal epithelial lesions of tyrosine fed rats. *Investigative Ophthalmology*, **14**, 937.
- GOLDSMITH, L.A. (1975) Hemolysis and lysosomal activation by solid state tyrosine. *Biochemical and Biophysical Research Communications*, **64**, 558.
- GOLDSMITH, L.A. (1976) Haemolysis induced by tyrosine crystals. *Biochemical Journal*, **158**, 17.
- GOLDSMITH, L.A. (1977) Molecular biology and molecular pathology of a newly described molecular disease—tyrosinemia II (The Richner-Hanhart syndrome). *Experimental Cell Biology*, in press.
- GOLDSMITH, L.A., KANG, E., BIENFANG, D.C., JIMBOW, K., GERALD, P. & BADEN, H.P. (1973) Tyrosinemia with plantar and palmar keratosis and keratitis. *Journal of Pediatrics*, **83**, 798.
- GOLDSMITH, L.A. & REED, J. (1976) Tyrosine-induced eye and skin lesions. A treatable genetic disease. *Journal of the American Medical Association*, **236**, 382.
- GSCHNITT, F., KONRAD, K., HÖNIGSMANN, H., DENK, H. & WOLFF, K. (1975) Mouse model for protoporphyria. I. The liver and hepatic protoporphyrin crystals. *Journal of Investigative Dermatology*, **65**, 290.
- HILL, A. & ZALESKI, W.A. (1971) Tyrosinosis: Biochemical studies of an unusual case. *Clinical Biochemistry*, **4**, 263.
- HOLSTON, J.L., LEVY, H.L., TOMLIN, G.A., ATKINS, R.J., PATTON, T.H. & HOSTY, T.S. (1971) Tyrosinosis: A patient without liver or renal disease. *Pediatrics*, **48**, 393.
- IP, C.C.Y. & HARPER, A.E. (1973) Effects of dietary protein content and glucagon administration on tyrosine metabolism and tyrosine toxicity in the rat. *Journal of Nutrition*, **103**, 1594.
- KENNAWAY, N.G. & BUIST, N.R.M. (1971) Metabolic studies in a patient with hepatic cytosol tyrosine aminotransferase deficiency. *Pediatric Research*, **5**, 287.
- KOZIN, F. & MCCARTY, D.J. (1976) Protein adsorption to monosodium urate, calcium pyrophosphate dihydrate, and silica crystals—relationship to the pathogenesis of crystal-induced inflammation. *Arthritis and Rheumatism*, **19**, 433.
- MANDEL, N.S. (1976) The structural basis of crystal-induced membranous lysis. *Arthritis and Rheumatism*, **19**, 439.
- NASH, T., ALLISON, A.C. & HARINGTON, J.S. (1966) Physico-chemical properties of silica in relation to its toxicity. *Nature*, **210**, 259.
- RICH, L.F., BEARD, M.E. & BURNS, R.P. (1973) Excess dietary tyrosine and corneal lesions. *Experimental Eye Research*, **17**, 87.
- SANDBERG, H.O. (1975) Bilateral keratopathy and tyrosinosis. *Acta Ophthalmologica*, **53**, 760.
- SCHWEIZER, W. (1947) Studies on the effect of l-tyrosine on the white rat. *Journal of Physiology*, **106**, 167.
- SCRIVER, C.R. & ROSENBERG, L.E. (1973) *Amino Acid Metabolism and Its Disorders* p. 338. W. B. Saunders, Philadelphia.

- SELYE, H. (1971) Steroids influencing the toxicity of L-tyrosine. *Journal of Nutrition*, **101**, 515.
- TABACHNICK, J. & LABADIE, J.H. (1970) Studies on the biochemistry of epidermis. IV. The free amino acids, ammonia, urea, and pyrrolidone carboxylic acid content of conventional and germ-free albino guinea-pig epidermis. *Journal of Investigative Dermatology*, **54**, 24.
- WEISSMANN, G. & RITA, G.A. (1972) Molecular basis of gouty inflammation: Interaction of monosodium urate crystals with lysosomes and liposomes. *Nature New Biology*, **240**, 167.
- ZALESKI, W.A., HILL, A. & KUSHNIRUK, W. (1973) Skin lesions in tyrosinosis: response to dietary treatment. *British Journal of Dermatology*, **88**, 335.

News and Notices

Hospital Physicists' Association

The Hospital Physicists' Association is planning to hold a one-day meeting on 18 May, 1978, on: 'Ultraviolet Radiation and its Medical Applications', at Imperial College, London.

The meeting will cover topics such as the medical and biological effects of UVR and the production and measurement of UVR, together with papers on current research including PUVA, irradiation of tumours and personnel monitoring of exposure to environmental UVR. The meeting is open to dermatologists and physicists.

Further information may be obtained from: Dr B.L. Diffey, Medical Physics Department, Kent and Canterbury Hospital, Canterbury, Kent CT1 3NG.

Eighth Annual Conference on Immunopathology

The Eighth Annual Conference on Immunopathology will be held on 19-23 June at the State University of New York at Buffalo, Buffalo, New York under the sponsorship of the Departments of Microbiology and Dermatology. The major faculty for the course include Drs Ernst H. Beutner and Russell J. Nisengard and their associates from SUNY at Buffalo, Drs Tadeusz P. Chorzelski and Stefania Jablonska from Warsaw, Poland, Dr Beno Michel from Case Western Reserve University, Dr Thomas Provost from SUNY at Buffalo and Dr Jean-Claude Bystran from New York University. This course meets the criteria for Category I of the American Academy of Dermatology Continuing Medical Education award and of the Physician's Recognition Award of the AMA.

The course this year is for professional personnel interested in utilizing immunofluorescence. Tissue antibodies and immunopathology will be studied in sera, skin biopsies and kidney biopsies. Registration fee is \$275.00. For technicians accompanying professional personnel the registration fee will be \$180.00. For further information write to Mrs Gloria Griffin, 219 Sherman Hall, SUNY/Buffalo, Buffalo, New York 14214.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometer(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	¹³¹ I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

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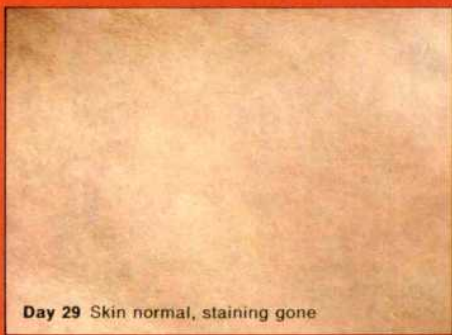
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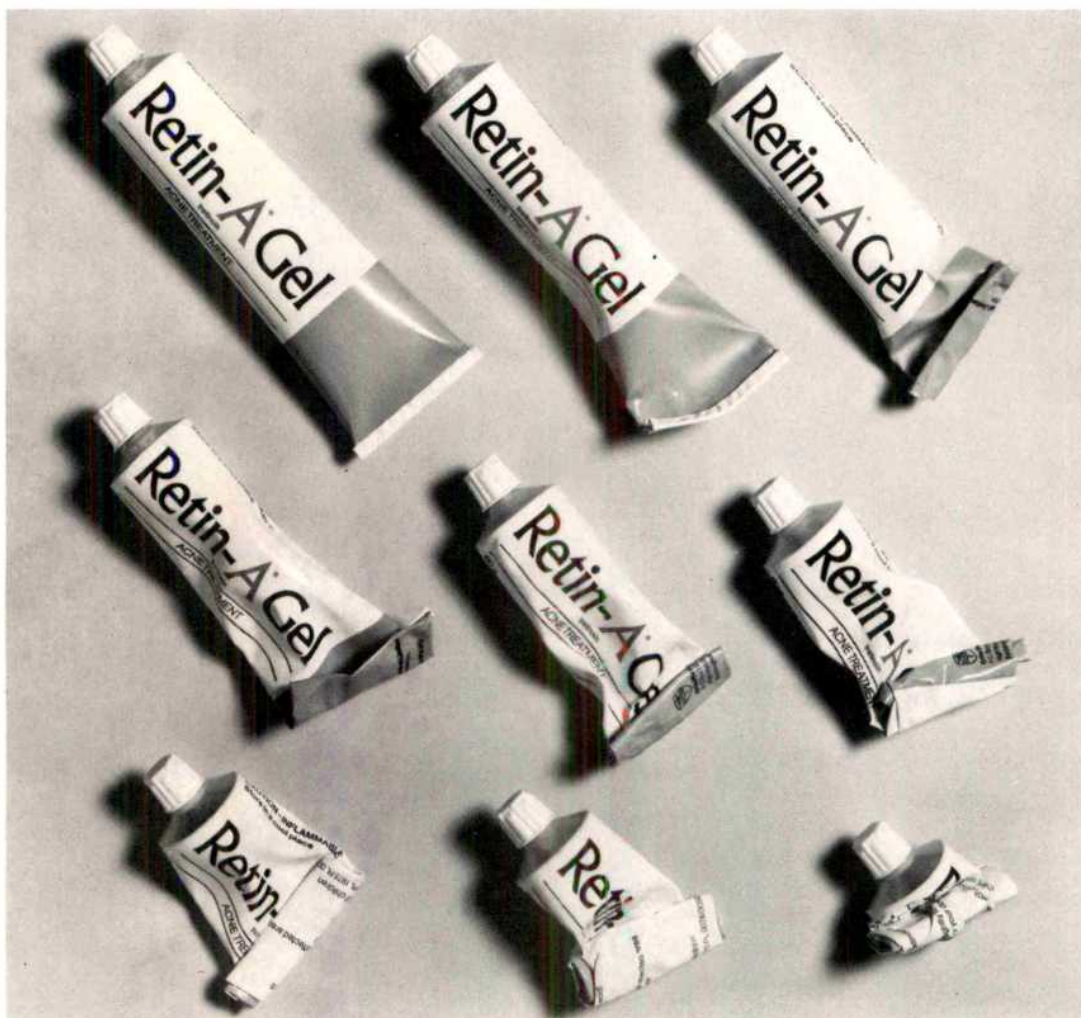
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1. Simpson, J. R. et al., (1973) Current Medical Research and Opinion, 1, 485. 2. Mills, O. H. et al., (1972) Archives of Dermatology, 106, 200. 3. Leyden, J. J. et al., (1974) Southern Medical Journal 67, 20. 4. Mandy, S. H. (1974) Cutis 14, 855-857.



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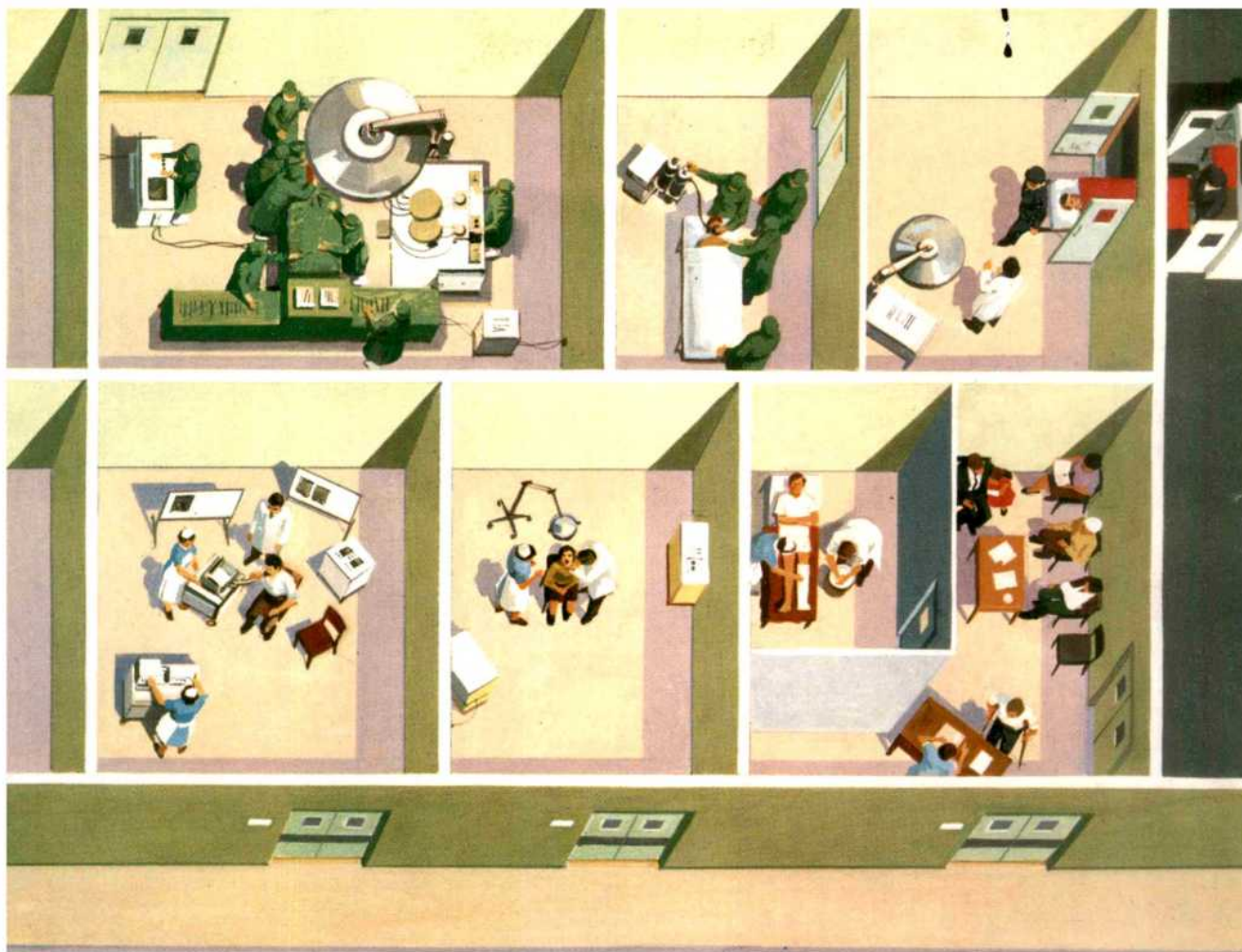
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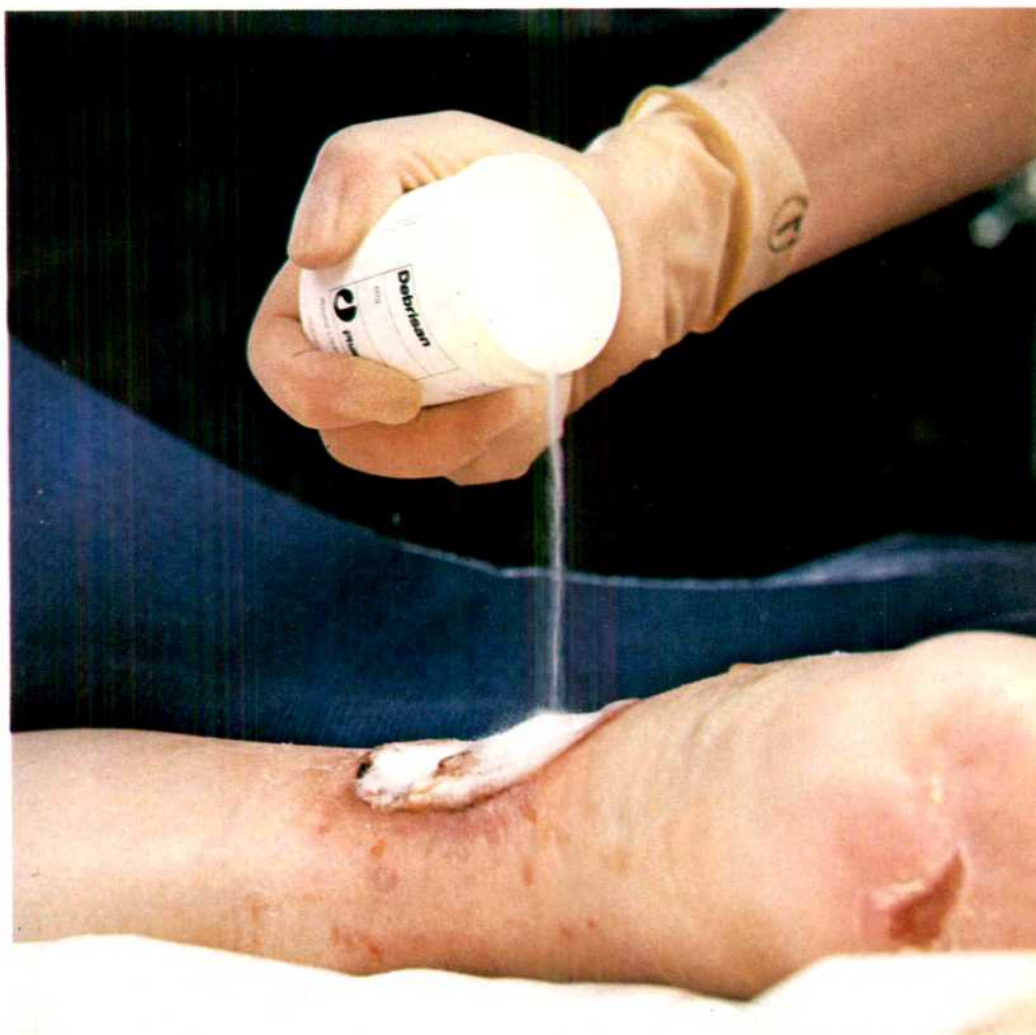
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⁵ Clin. Trials J. (1970) 7:368. ⁶ Clin. Trials J. (1970) 7:375. ⁷ Brit. J. Clin. Pract. (1971) 25:371. ⁸ Chemotherapy (1968) 17:1473.
⁹ Brit. J. Clin. Pract. (1970) 24:41. ¹⁰ Curr. Med. Res. Opin. (1975) 3:77. ¹¹ Scand. J. Infect. Dis. (1974) 6:273.
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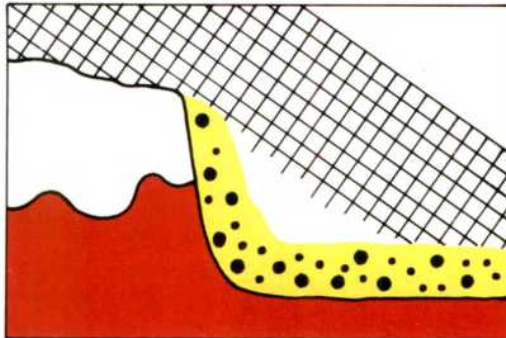
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sometimes painful



Top and bottom left

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Red: Oedema

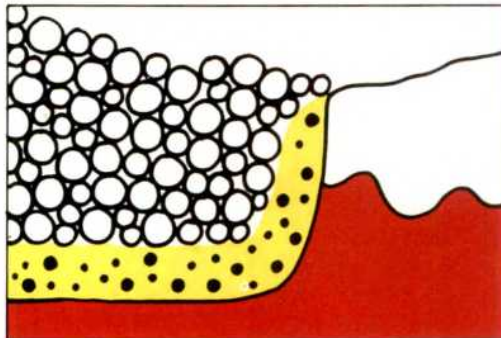
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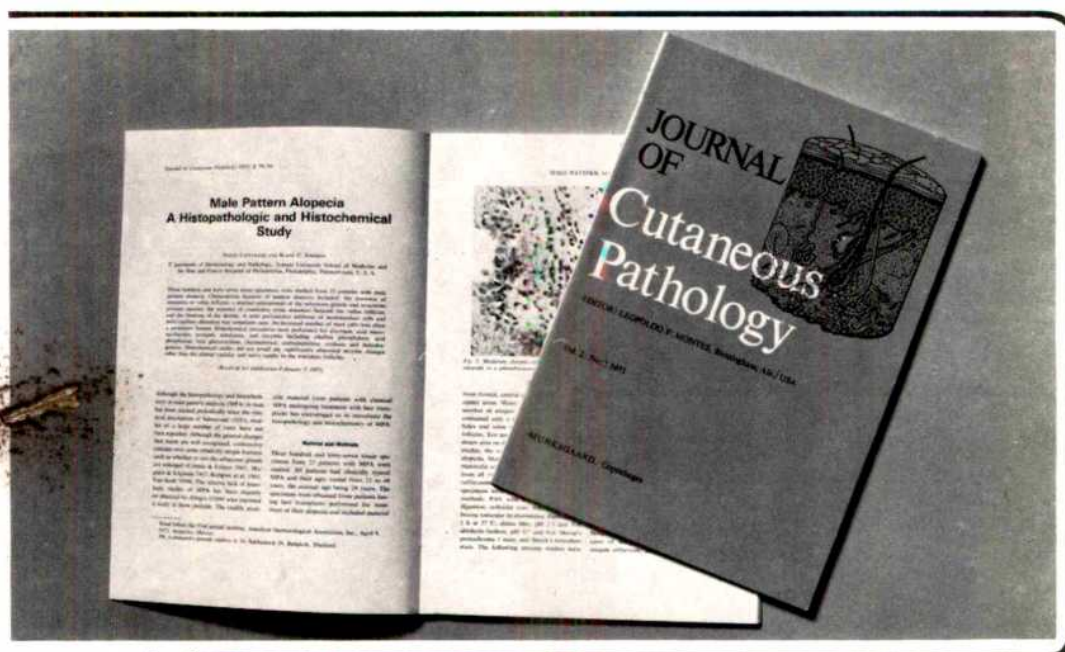
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Dermatology



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HISTOPATHOLOGY OF THE SKIN

Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

More than 20 diseases not mentioned in the fourth edition are described in this edition. Some of them are newly recognized entities, such as disseminated superficial actinic porokeratosis, perforating folliculitis, reactive perforating collagenosis, angiolymphoid hyperplasia with eosinophilia, infantile digital fibromatosis, trichilemmoma, and malignant eccrine poroma. Also several previously known diseases because of their increased interest for the dermatologist have been incorporated, among them regional enteritis (Crohn's disease), Tangier disease, lipogranulomatosis (Farber's disease), and generalized eruptive histiocytoma.

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Controlled study of PUVA and adjunctive topical therapy in the management of psoriasis

WARWICK L. MORISON, JOHN A. PARRISH AND
THOMAS B. FITZPATRICK

Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts,
02114, U.S.A.

Accepted for publication 8 August 1977

SUMMARY

In a random study of 116 patients with psoriasis vulgaris, oral psoralen photochemotherapy (PUVA) used alone was compared to PUVA plus adjunctive topical therapy with tar, dithranol, or topical corticosteroids. PUVA plus topical corticosteroids produced more rapid clearing of psoriasis but despite maintenance therapy, the frequency of recurrences of the disease during the early phase of follow-up was significantly higher with that treatment, as compared to all other treatments. Dithranol plus PUVA also cleared psoriasis quicker than PUVA alone but patient acceptability for that regimen was low. The addition of tar to PUVA therapy appeared to have little influence on results.

Photochemotherapy using oral 8-methoxypsoralen and subsequent exposure to longwave ultraviolet radiation (PUVA) has been reported as a satisfactory treatment for psoriasis (Parrish *et al.*, 1974; Wolff *et al.*, 1976; Melski *et al.*, 1977). In order to assess accurately the efficacy and safety of PUVA therapy, controlled studies with psoralen and UV-A radiation to date have avoided any adjunctive treatment. However, when the treatment is more widely used, it is likely that it will often be used in combination with existing modalities (e.g. tar, corticosteroids).

The present study had two objectives. Firstly, to determine whether there are any benefits or problems arising from combining conventional topical agents with PUVA. Secondly, to assess whether an initial outpatient course of conventional topical treatment aimed at clearing the disease, followed by maintenance PUVA therapy, had any advantages or disadvantages over the use of PUVA as both a clearance and a maintenance treatment. Tar, dithranol, and corticosteroids, the three most commonly used topical agents, were chosen for evaluation. After clearance of psoriasis to less than 1% body involvement, the study was designed to include a 4-month follow-up on maintenance PUVA therapy without any topical therapy.

METHODS

Patients

One hundred and sixteen patients were entered into the study; all patients were treated as out-

patients. The mean age of the group was 40 years and the mean duration of their psoriasis was 16 years. The study was limited to patients with psoriasis vulgaris who were not being treated with systemic corticosteroids or cytotoxic agents. Pregnancy and aphakia were additional contraindications to treatment. The area of skin involved by disease ranged from 10% to 90% with an average involvement of 35% for the group (rule of 9 for large areas; palm equals 1% body surface area for small areas). At the time of evaluation, 84% of the patients had psoriasis of the scalp, 68% had intertriginous involvement, and 12% had active arthritis of the psoriatic type.

Patients whose disease state and motivation made them acceptable for this study were randomly allocated to one of the treatment groups. The control patients were not randomly allocated but instead were consecutive patients giving a history of no treatment for their disease in the 3 months immediately preceding entry into the study.

Design of the study

One hundred and sixteen patients were divided into three groups (Table 1):

TABLE 1. Design of study

Group 1 (58 patients)
PUVA and concurrent adjunctive topical therapy
1A: PUVA plus dithranol
1S: PUVA plus steroids
1T: PUVA plus tar
Group 2 (40 patients)
Topical therapy commenced 6 weeks before PUVA
Then PUVA plus topical therapy until clear of psoriasis
2A: dithranol for 6 weeks, then PUVA plus dithranol
2S: steroids for 6 weeks, then PUVA plus steroids
2T: tar for 6 weeks, then PUVA plus tar
Control Group (18 patients)
PUVA therapy plus lubricants only

All topical medications used daily.
PUVA given twice weekly.

Group 1. Fifty-eight patients received PUVA therapy plus a topical agent during the clearance phase, both treatments being commenced at the same time. This group was subdivided according to the topical agent used.

1A. Twenty patients were treated with dithranol applied to the body and scalp at night and removed in the morning. The dithranol preparation used depended on the clinical appearance of the psoriasis and the response to treatment, and the strength of the preparation was constantly adjusted to maximize the therapeutic benefit. In increasing order of potency the preparations used were: 0.25, 0.5 and 1% dithranol ointment, 0.1, 0.2 and 0.4% dithranol in Lassar's paste (Lasan 1, 2 and 4). Dithranol 0.4% in a pomade base (Lasan pomade) was used for scalp treatment.

1S. Nineteen patients were treated with fluocinolone acetonide under plastic occlusion to the body and scalp at night. For the body 0.025% cream or ointment were used and for the scalp 0.01% lotion.

1T. Nineteen patients were treated with a tar preparation applied to the body and scalp at night. Estar (biologically equivalent to 5% coal tar, in a hydro-alcoholic gel) was used on the trunk and limbs and a pomade base, containing solution of coal tar 12% and salicylic acid 2%, was applied to the scalp.

Group 2. Forty patients received 6 weeks of topical therapy alone (without any PUVA therapy) with the aim of clearing the disease to less than 1% body surface involvement. At the end of that period, or earlier if the disease was cleared, PUVA therapy was commenced. Patients not clear at 6 weeks continued to use topical therapy in addition to PUVA. This group was also divided into three subgroups and each received a different topical agent. The same agents and regimens were used as in Group 1: dithranol (19 patients), fluocinolone acetonide (19 patients), and tar (2 patients).

Control group. Eighteen patients were treated with PUVA therapy used alone. Except for the use of lubricants as required to relieve dryness, this group received no topical treatment.

All patients with scalp involvement used Sebulex shampoo (surface active cleansers and wetting agents, sulphur 2%, salicylic acid 2%) daily. During the maintenance phase of treatment, patients were allowed to continue topical therapy on the scalp. No other topical therapy was used after clearance; PUVA alone was used for maintenance and the treatment of any subsequent recurrence of the disease.

PUVA therapy

8-Methoxypsoralen (0.6 mg/kg body weight) was ingested 2 h before each light exposure. Total body exposure was given in a high intensity UV-A (320–400 nm) system (Sylvania), the characteristics of which have been described (Parrish *et al.*, 1974; Levin & Parrish, 1974). The starting dose of UV-A (1.5–6.5 J/cm²) was based on the patients' skin type (I–VI) as determined by previous sunburn and suntan history and physical examination. (National Academy of Sciences, 1976.) Increments of 0.5–1.0 J/cm² were given with each treatment. During the clearance phase, treatments were given twice weekly but never less than 48 h apart. Once the psoriasis had cleared, maintenance treatments were commenced at the final clearance dose of UV-A on a schedule of: 4 treatments at weekly intervals followed by 4 treatments at fortnightly intervals and then monthly. A relapse of psoriasis was defined as psoriasis involving >5% body surface; if this occurred the schedule reverted to twice weekly treatment.

Evaluation and laboratory investigations

During the clearance phase physician follow-up was at 2–3 week intervals or more frequently if problems arose. Careful attention was paid not only to progress of the disease but also to patient compliance with the topical regimen and their comments about the regimen. During the maintenance phase patients were seen monthly.

Before starting therapy, all patients had a complete blood count, serum creatinine, BUN, SGOT, LDH, alkaline phosphatase and urinalysis. These studies were repeated after 6 months of therapy. In addition, a pretreatment ophthalmological examination was performed, to be repeated at yearly intervals.

RESULTS

There was no significant difference between any of the treatment groups or the control group in terms of age, sex, skin type, extent of psoriasis, previous systemic cytotoxic therapy, and previous hospitalization. The control group differed in that they had not had any therapy for the 3 months prior to entry into the study. The first two patients entered into the 2T schedule (tar for 6 weeks and then tar and PUVA) showed no change at all in the appearance or extent of their psoriasis over the initial 6-week treatment period. Staining of their skin supported their claim that they had used the treatment. No further patients were entered into this group.

TABLE 2. Results of topical therapy: compliance varied inversely with satisfaction rate, e.g. dithranol had a high dissatisfaction rate and low compliance. Only 11% (4/38) patients in Group 2 achieved clearance with topical therapy

Patients	Dithranol	Steroid	Tar
% used treatment according to regimen	67	74	95
% dissatisfied with treatment regimen	72	63	48
Response in Group 2 before starting PUVA			
% cleared	16	5	
% improved	68	69	100
% no change	11	5	
% worse	5	21	

Table 2 outlines the results achieved with topical therapy. Overall, 76% of the patients claimed that they used the topical agent exactly as required by their schedule while most of the other patients stated that their usage rate averaged three times a week or more. Many patients expressed dissatisfaction with the topical therapy, dithranol being the least popular. As would be expected, compliance varied inversely with satisfaction (e.g. the dithranol group had a high dissatisfaction rate and a low compliance). In the dithranol treated group, in addition to the usual complaints of staining of the skin and clothing, difficulty in removing the applied paste and skin irritation, two patients developed extensive chemical burns from the dithranol paste which resulted in a Köbner response and an extension of their psoriasis. The main complaints in the steroid treated groups were of discomfort and the inconvenience of occlusive therapy as well as the time involved in carrying out the daily treatment. The tar preparation was the most acceptable of the three treatments, the only complaints being dryness and occasional stinging sensations. It can be seen that only a small percentage of Group 2 managed to clear themselves within the 6 weeks of topical therapy alone, but the majority did experience some improvement.

PUVA therapy, with or without topical agents, achieved clearance of psoriasis to less than 1% body involvement in 92% of the 116 patients. This required a mean of 17 PUVA treatments over 69 days and a final clearance UV-A dose of 15 J/cm². Table 3 outlines the results in the individual treatment groups. Group 1 patients, who received topical therapy in conjunction with PUVA, cleared more quickly with fewer treatments and at a lower final UV-A dose as compared to the control patients. Group 2 patients, because they had 42 days of topical therapy before commencing PUVA treatments, took longer to clear as compared with Group 1 patients, but similarly required fewer PUVA treatments and a lower final UV-A dose when compared with the control group.

The response to therapy of psoriatic involvement of the scalp, intertriginous areas and nails in patients who cleared to less than 1% body involvement is shown in Table 4. Ninety percent of the 77 patients using topical therapy on their scalp (Groups 1 and 2 patients) cleared the psoriasis in this area by the time their body psoriasis had cleared. This response rate is significantly different ($P < 0.01$) from that of the control group in which only 2 of the 13 (15%) patients with scalp involvement cleared. Those two patients had only minimal scalp involvement, limited to the margins. When reviewed 4 months later, of the 71 patients who did clear their scalp of psoriasis 41% had remained clear without any topical therapy. The remaining 59% continued to use topical therapy for the scalp. Seventy-five percent of the 45 patients with involvement of the intertriginous areas cleared in those areas, but no

TABLE 3. Patients cleared to <1% body involvement: concurrent PUVA and topical therapy (Group 1) produced clearance of psoriasis quicker with fewer PUVA treatments and at a lower final UV-A dose as compared to PUVA alone (control group)

	% Patients (and no.) cleared	No. days to clear	No. treatments to clear	Final clearance Dose UV-A in J/cm ²	
				Mean	Range
Controls	100 (18/18)	97	23	17	7-36
Group 1					
A	95 (19/20)	60	15	12	4-35
S	100 (19/19)	59	14	11	3-25
T	84 (16/19)	69	18	16	6-29
Group 2					
A	84 (16/19)	108	14	13	5-27
S	89 (17/19)	108	15	12	0-18
T	100 (2/2)	112	18	16	12-20

appreciable differences were found between the treatment groups. Forty-six percent of the 79 patients with nail disease cleared by the time of the four-month review. Nail disease was slower to respond than psoriasis of the skin and only a few patients had cleared by the time they had reached less than 1% body involvement. Nail disease was classified according to the predominant type present in each patient (pitting, onycholysis, dystrophy) but no significant differences were seen in the response rates of the different types. In addition, the response of the nail disease did not differ between the treatment groups. All patients with psoriatic arthritis cleared their psoriasis, but none of them noted any symptomatic alteration in the severity of their arthritis during the course of treatment.

TABLE 4. Response in special areas in patients cleared to <1% body involvement. 90% patients using topical therapy on scalp (Group 1 and 2) cleared as compared to 15% of control group ($P < 0.01$). Response rates for intertriginous and nail disease were similar for all groups

	Patients showing clearing of:		
	Scalp	Intertriginous areas	Nails
Controls	2/13	7/10	3/15
Group 1			
A	11/13	2/4	8/14
S	15/17	6/7	5/14
T	14/16	7/8	8/14
Group 2			
A	13/13	3/7	7/11
S	14/16	8/9	3/9
T	2/2		2/2

When patients who had cleared to less than 1% body involvement were fully reviewed 4 months after the time of their clearing, the only important difference noted between the groups was in the frequency of exacerbations of the disease. Patients treated with topical steroids (Groups 1S and 2S) had a significantly greater incidence of relapse of their disease as compared to all other patients ($P < 0.005$) (Fig. 1). The clinical picture seen in most of these exacerbations was that of an acute, rapidly spreading psoriasis. Many of these patients initially presented with stable, plaque-form psoriasis and the combined PUVA and topical steroid therapy appeared to convert their disease into an unstable and aggressive form. Furthermore, in some cases the exacerbation was more difficult to treat than the original disease and required a greater number of PUVA treatments and a higher final UV-A dose.

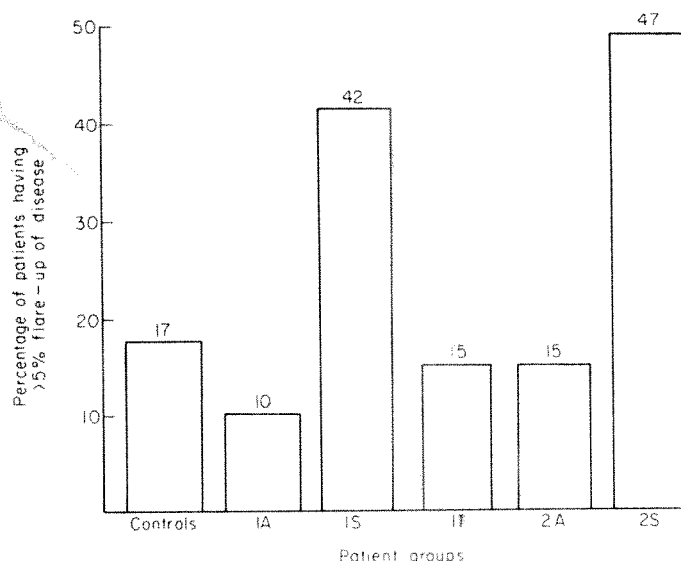


FIGURE 1. Disease exacerbations during first 4 months of maintenance therapy. Patients treated with topical steroids 1S and 2S had greater frequency of exacerbations as compared to all other patients ($P < 0.005$).

As has been previously reported, nausea, erythema, and pruritus were seen in some patients after PUVA therapy but no significant differences in the frequency of these side effects were found between the treatment groups. The LDH was the only laboratory investigation to show a shift outside the normal range in any patient. Five patients showed this change with a mean rise of 41 units and the highest level recorded was 186 units (normal range up to 120 units). Three months later the values had returned to normal in three of these patients. These patients were otherwise well and no explanation has been found for these abnormal values. There was no general trend for LDH values to rise.

DISCUSSION

PUVA has now been established as an effective outpatient therapy in the management of psoriasis (Parrish *et al.*, 1974; Wolff *et al.*, 1976; Melski *et al.*, 1977). The present study was designed to explore the interaction between PUVA therapy and existing topical treatments for psoriasis in terms of the benefits and the problems that may result from such combination therapy. All patients in this study were treated as outpatients and the question of cooperation in using the topical regimen must be considered in evaluating the results. Publicity about the PUVA program and the long waiting list for

entry appear to have combined to provide a very cooperative patient population in this centre. The aims of the study were fully explained to each patient at the initial evaluation visit and constant reinforcement was provided throughout the study. For these reasons, a high compliance rate for the topical regimen was achieved and even patients who expressed dissatisfaction continued to co-operate.

No advantage was gained by giving patients a preliminary outpatient course of conventional topical therapy (Group 1 patients). Only 10% of the patients cleared their psoriasis and the majority were unhappy using this pre-PUVA course of therapy. Furthermore, this group subsequently required the same number of PUVA treatments to clear their psoriasis as did the PUVA plus concurrent topical therapy group. Therefore, the pre-PUVA course of topical therapy merely lengthened the clearing phase by six weeks without any real benefit to the patients.

By comparison, patients who used topical agents and PUVA, both treatments being commenced at the same time, were not as reluctant to use the adjunctive topical treatment and did clear earlier and at a lower final mean UV-A dose than patients in the control group using PUVA alone. To balance this apparent advantage, however, several disadvantages emerged. There was resistance to using any topical treatment on an out-patient basis and undoubtedly the topical regimens were both time-consuming and unpleasant for the patients. The combination of topical steroids and PUVA therapy resulted in a greatly increased relapse rate in the immediate post-clearance period. Of more concern was the fact that most of these relapses consisted of an aggressive, unstable form of psoriasis which was difficult to control with PUVA therapy. The most obvious explanation for these exacerbations is that they were precipitated by withdrawal of topical steroids. However, it is possible that the combination of steroids and PUVA produce an adverse effect on psoriasis and should not be used together in the therapy of this disease. Dithranol combination therapy was not without its problems. It was the least acceptable of the topical treatments, and two patients did develop extensive Köbner phenomena following dithranol-induced burns. Refined tar as used in this study had the advantage of being the most acceptable to the patients of the three topical agents, but it appeared to have little effect on the disease.

PUVA therapy has not been found to be useful in the treatment of scalp psoriasis because hair scatters and absorbs most of the incident UV-A. This study clearly shows that adjunctive topical therapy with PUVA is better than PUVA therapy alone for the treatment of the scalp. Furthermore, at the 4-month follow-up, 41% of the patients who had cleared their psoriasis were still clear without any further topical therapy. It is possible that the small amount of radiation penetrating the hair, while being insufficient to clear the disease, may be adequate for maintenance of a clear state.

Psoriatic nail disease cleared in 46% of the affected patients by the time of the four-month post-clearance follow-up. Disease at that site takes longer to clear than does psoriasis elsewhere and possibly more patients would have cleared with a longer follow-up. Psoriatic arthritis did not alter during therapy, but this is not surprising as little incident UV-A would be expected to reach the synovium of most joints.

All treatment was conducted on an outpatient basis since PUVA therapy does not usually require hospitalization. If the adjunctive topical therapy had been conducted on an inpatient basis, it could be expected that the response to this part of the treatment would have improved. However, the long-term results would have only improved in the dithranol group because the disadvantages of the steroid-PUVA combination and the lack of response to tar would, presumably, still be observed.

This study indicates that adjunctive topical therapy has a place in combination with PUVA in the treatment of scalp psoriasis. In addition, it may have a place in the treatment of resistant local areas, but the three topical agents investigated in this study have some disadvantages. Steroids do not appear to interact well with PUVA, dithranol had the risk of chemical burns with subsequent Köbner-phenomenon and tar did not appear to be very effective.

REFERENCES

- Halocarbons: Environmental effects of chlorofluoromethane release, (1976) National Academy of Sciences, p. E-2.
- LEVIN, R.E. & PARRISH, J.A. (1974) Phototherapy of vitiligo. *Lighting Design and Application*, **4**, 38.
- MELSKI, J.W., TANENBAUM, L., PARRISH, J.A., FITZPATRICK, T.B., BLEICH, H.L. and 28 participating investigators (1977) Oral methoxsalen photochemotherapy for the treatment of psoriasis: a cooperative clinical trial. *Journal of Investigative Dermatology*, **68**, 328.
- PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, L. & PATHAK, M.A. (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. *New England Journal of Medicine*, **291**, 1207.
- WOLFF, K., FITZPATRICK, T.B., PARRISH, J.A., GSCHNAIT, F., GILCHREST, B., HONIGSMANN, H., PATHAK, M.A. & TANENBAUM, L. (1976) Photochemotherapy for psoriasis with orally administered methoxsalen. *Archives of Dermatology*, **112**, 943.

Psoriasis treated with clobetasol propionate and photochemotherapy

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SUMMARY

Comparable lesions in 16 patients with chronic plaque psoriasis were treated with clobetasol propionate or placebo ointments for 1 week before beginning photochemotherapy. The greater improvement of the steroid treated lesions was maintained after the ointment was withdrawn and there was a mean period of 31 days before those treated with placebo showed a comparable response to photochemotherapy. By the end of the study 8 patients were completely free of psoriasis, healing being achieved after a mean of 7.2 photochemotherapy treatments on the steroid treated lesions compared with 13.5 where a placebo ointment was used. No patient had any adverse reaction to treatment.

A pilot study was undertaken to see if pre-treatment for 1 week with a potent topical corticosteroid altered the response of psoriasis to photochemotherapy.

METHOD

Patients with chronic plaque psoriasis, which was unresponsive to other treatments, were treated with photochemotherapy as previously described (Lakshminpathi *et al.*, 1977). All except one, who was initially treated in hospital, were out-patients. At the preliminary visit, the patient's minimal photo-toxic dose was determined, and a twin-pack was issued which consisted of two identical-looking tubes of ointment, one containing clobetasol propionate 0.05% ('Dermovate') and the other unmedicated ointment base. The ointments were used twice daily on symmetrical, equally affected, plaques. Active and placebo ointments, so labelled that neither the patients nor the doctor was able to distinguish between them, were randomly allocated to right and left lesions. After using the ointments for 1 week the patient attended the clinic again and any difference between the two, previously similar, plaques was recorded. No further topical steroid was used during the study.

Photochemotherapy was then begun. Prior to each subsequent exposure the pre-treated lesions were characterized as 'healed', 'improved', 'static' or 'worse'. When the study was terminated the code was broken and any difference noted in the response to photochemotherapy of those lesions treated with the active and placebo ointments.

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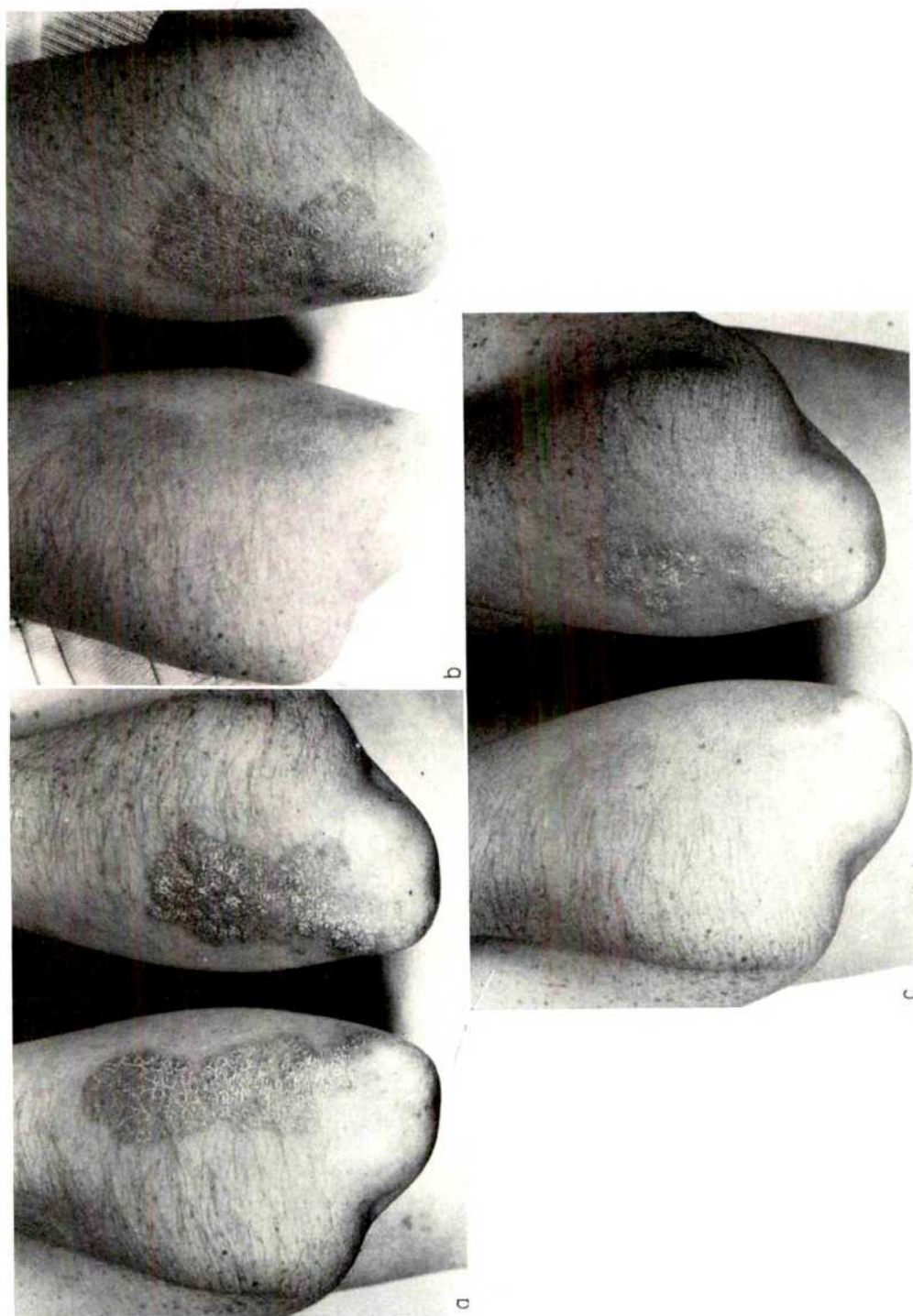


FIGURE 1. Comparable plaques of psoriasis. (a) Before treatment. (b) After 1 week's treatment with clobetasol propionate (right elbow) and placebo ointments (left elbow). (c) Before treatment. (d) After 8 weeks' use of photochemotherapy only (20 treatments).

RESULTS

Sixteen patients were studied. At the end of the week's application of clobetasol propionate and unmedicated base all had shown a greater improvement on one lesion than the other. When the trial was completed it was found that in each case this greater improvement was associated with the use of the active ointment.

One patient's photochemotherapy was carried out using topical 8-methoxypsoralen; the other 15 were given the drug orally. The duration of treatment ranged from 4 to 17 weeks (median 10 weeks). Fifteen of the 16 patients had an unequal response to photochemotherapy, in the one exception both lesions were similarly improved (but not healed) after 4 weeks. All the other patients had a more rapid and/or greater response on the lesion pre-treated with clobetasol propionate (Fig. 1). In the 8 patients in whom both lesions healed the mean number of exposures to photochemotherapy to achieve healing was 7.2 on the steroid treated lesion compared with 13.5 on the lesion treated with unmedicated ointment. Fourteen patients had equally healed or improved lesions by the end of the trial. The time taken for the placebo treated lesion to heal to the same extent as the lesion treated with steroid ranged from 9 to 74 days (mean 31.5 days).

On one occasion in each of three of the patients the pre-treated lesions were characterized as having deteriorated since their last attendance. In two of these this happened while photochemotherapy was still being given on alternate days; the third patient, who responded to treatment only slowly, had her relapse when she was attending for maintenance photochemotherapy once weekly. The relapses were assessed as slight or moderate and in all three patients were more marked on one of the pair of pretreated lesions. At the end of the trial the more stable lesion was found to be that to which steroid ointment had been applied.

In one further patient, a 31-year-old woman who had psoriasis since she was 13, the steroid treated plaque was completely healed after her sixth photochemotherapy treatment. By contrast, her other areas of involved skin were scarcely improved at all despite 20 treatments over 6 weeks. Conventional ultraviolet light and tar treatment was then substituted for photochemotherapy and she gradually showed some improvement. The clobetasol propionate treated area remained clear throughout.

DISCUSSION

This study indicates that the short-term pre-treatment of psoriasis with clobetasol propionate ointment reduces the number of times photochemotherapy needs to be used. Morison, Parrish & Fitzpatrick (1978) have made a similar observation. However, this group found that when their patients were reviewed after 4 months nearly half of those who had been treated with both topical steroids and photochemotherapy had relapsed. They also noted that the relapses were of a more aggressive, unstable, nature, less responsive to photochemotherapy.

The discrepancy between our findings might be explained by the differences in the topical steroid regimes used, as the follow-up periods did not greatly differ. However, the use of a short (1 week) pre-treatment course of ointment, without occlusive dressings, as in our study, may have very different consequences from the long (6 weeks) intensive (whole body occlusion) therapy used in theirs. The fact that different corticosteroids, clobetasol propionate and fluocinolone acetonide, were used may also have a bearing on the variable findings.

The possibility that the number of treatments with, and the quality of response to, photochemotherapy may be favourably influenced by the short-term pre-treatment of psoriasis with topical steroids warrants further investigation.

REFERENCES

- LAKSHMIPATHI, T., GOULD, P.W., MACKENZIE, L.A., JOHNSON, B.E. & FRAIN-BELL, W. (1977) Photochemotherapy in the treatment of psoriasis. *British Journal of Dermatology*, **96**, 587.
- MORISON, W.L., PARRISH, J.A. & FITZPATRICK, T.B. (1978) Controlled study of PUVA and adjunctive topical therapy in the management of psoriasis. *British Journal of Dermatology*, **98**, 125.

Photoprotection of the eye in PUVA therapy

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Photochemotherapy with psoralens, now widely used in the therapy of psoriasis, vitiligo and mycosis fungoides, has been proposed to have the capacity, during long-term therapy, to induce ocular damage, e.g. cataract. This view is based on animal experiments (Cloud, Hakim & Griffin, 1960, 1961; Freeman & Troll, 1969) and as a consequence adequate protection of the eyes has been recommended for patients on PUVA treatment.

During therapeutic irradiation eye protection is usually provided by small black non-transparent glasses but the problem is which type of sun glasses the patient should wear for the rest of the day of treatment.

The purpose of this paper was to investigate different sun glasses on the market with regard to their transmission of longwave ultraviolet light around 365 nm (UVA) where the maximal action spectrum for psoralens is found *in vivo* (Pathak, Krämer & Fitzpatrick, 1974).

MATERIAL AND METHODS

The ultraviolet light source used was a Black-Ray B-100 Lamp (Ultraviolet Products Inc., U.S.A.) providing longwave ultraviolet radiation ranging from 350 to 380 nm, intensity maximum at 365 nm. The intensity of the lamp was 15 mW/cm², as measured with a PUVA-meter (Herbert Waldmann Werk für Lichttechnik) at a distance of 20 cm from the lamp. The glasses being investigated were placed just in front of the PUVA-meter detector. The PUVA-meter used has its greatest sensitivity in the UVA region with a maximum around 365 nm.

Different sun glasses were investigated. (a) Spectacles of the trademark Noir (U.S.A.), (b) Blak-Ray Contrast Control Spectacles Model No. UVC-303 (Ultraviolet Products Inc.), (c) 8 sun glasses of the trademark Polaroid (U.S.A.) with differences in the colour of the glasses, *viz.* brown, grey and green, (d) glasses which darken with light intensity, Umbramatic (Zeiss), and (e) 10 different unsorted cheap sun glasses available in department stores.

RESULTS

The results are given in Table 1. Ordinary unsorted cheap sun glasses give a very unreliable protection against UVA, the transmission varying from about 13 up to 67%. The glasses designed to darken with light intensity (Umbramatic) showed a transmission of 17% after maximal darkening. All the other glasses (Noir, Blak-Ray and Polaroid) provided excellent protection against UVA with no measurable transmission during the experimental conditions used.

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TABLE 1. Transmission of UVA by different glasses when investigated with a Blak-Ray B-100 Lamp at a distance of 20 cm from the lamp. Percent values expressed relative to the control value for unfiltered radiation at this distance 15 mW/cm²

Investigated glasses	Transmission (UVA)	
	mW/cm ²	%
(a) Noir	0	0
(b) Blak-Ray UVC-303	0	0
(c) Polaroid (8 different models)	0	0
(d) Umbramatic	2.5	16.7
(e) Unsorted sun glasses 1	2.0	13.3
2	2.2	14.7
3	2.3	15.3
4	2.5	16.7
5	3.3	22.0
6	3.6	24.0
7	4.0	26.7
8	4.0	26.7
9	7.0	46.7
10	10.0	66.7

DISCUSSION

The possibility of ocular damage caused by photochemotherapy with psoralens has been shown in animal experiments (Cloud *et al.*, 1960, 1961; Freeman *et al.*, 1969) but the doses used in such experiments have been unphysiological and more than 100-fold greater than those in therapeutic use. Doses comparable to therapeutic doses in man have not induced any eye injury (Fitzpatrick *et al.*, 1966; Pathak *et al.*, 1974; El Mofty, 1977). There are no reports of cataract formation or other ocular damage in patients with vitiligo who have been treated with large doses of 8-methoxypsoralen



FIGURE 1. Glasses with an excellent protection against UVA with no measurable transmission during the experimental conditions used. A and B have the advantage of side protection. (A) Noir, (B) Blak-Ray UVC-303 and (C) two different Polaroid glasses.

or trimethylpsoralen for periods as long as 8 or 12 years (Fitzpatrick *et al.*, 1966; Pathak *et al.*, 1974; El Mofty, 1977).

However, the present approach amongst investigators managing PUVA treatment is to prescribe not only complete protection of the eyes during therapeutic irradiation but also the use of sun glasses during the rest of the day when treatment has been given. The present study shows that a safe and simple recommendation to the patients is the use of the easily available Polaroid-glasses. The trademark Polaroid should be found on the spectacles; this is emphasized as more or less inferior imitations are found on the market. When effective side protection is desirable, glasses such as Noir or Blak-Ray should be recommended (Fig. 1).

REFERENCES

- CLOUD, T.M., HAKIM, R. & GRIFFIN, A.C. (1960) Photosensitization of the eye with methoxsalen. I. Acute effects. *Archives of Ophthalmology*, **64**, 346.
- CLOUD, T.M., HAKIM, R. & GRIFFIN, A.C. (1961) Photosensitization of the eye with methoxsalen. II. Chronic effects. *Archives of Ophthalmology*, **66**, 689.
- EL MOFTY, A.M. (1977) Chronic retrospective ocular study of patients receiving oral 8-methoxypsoralen and exposed to solar irradiation for the treatment of vitiligo. Paper presented at the Dermatological and Venereological Conference in Cairo, Feb 20-24, 1977. Abstracts, 101.
- FITZPATRICK, T.B., ARNDT, K.A., EL MOFTY, A.M. & PATHAK, M.A. (1966) Hydroquinone and psoralens in the therapy of hypermelanosis and vitiligo. *Archives of Dermatology*, **93**, 589.
- FREEMAN, R.G. & TROLL, D.J. (1969). Photosensitization of the eye by 8-methoxypsoralen. *Journal of Investigative Dermatology*, **53**, 449.
- PATHAK, M.A., KRÄMER, D.M. & FITZPATRICK, T.B. (1974) Photobiology and photochemistry of furocoumarins (psoralens). In: *Sunlight and Man* (ed. T.B. Fitzpatrick, M.A. Pathak, L.C. Harber, M. Seiji and A. Kukita), pp. 335-368. University of Tokyo Press.

Dose-effects of 8-methoxypsoralen and UVA in cultured human lymphocytes

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SUMMARY

Cultures of human lymphocytes were incubated with 8-methoxypsoralen (8-MOP) and irradiated with long wave ultraviolet light (UVA). Subsequently the mitogenic response to PHA was determined by ^3H -thymidine incorporation. The minimal effective dosages of drug and light for the partial suppression of lymphocyte PHA-responsiveness were found to be $0.1 \mu\text{g/ml}$ 8-MOP and 0.5 J/cm^2 UVA. While an increase in the concentration of 8-MOP revealed a comparatively small rise in photo-toxicity, a marked inhibition of ^3H -thymidine uptake was obtained by increasing the UVA dosage. Cell viability of non-stimulated lymphocytes was similarly affected by the combined treatment.

Peripheral human lymphocytes have been shown to be sensitive to the *in vitro* effects of 8-methoxypsoralen (8-MOP) and UVA (Swanbeck *et al.*, 1975). The treatment with both 8-MOP and UVA significantly inhibited the subsequent stimulation of DNA synthesis by phytohaemagglutinin (PHA) (Scherer *et al.*, 1977). Recently, evidence has been presented indicating that cellular immune reactions play a role in the aetiology of psoriasis (Ortonne *et al.*, 1977; Glinski *et al.*, 1977). Since during PUVA treatment considerable amounts of UVA energy are absorbed in the papillary layer of the skin, the sensitivity of lymphocytes located in the dermal infiltrates of psoriasis could well be important in the mechanism of action of PUVA.

In addition, it is well known that the effectiveness of this treatment strongly depends upon the proper dosage of 8-MOP as well as of UVA. Any conclusions concerning the results in the mechanism of PUVA therefore have to take into account the applied doses of 8-MOP and UVA. We have investigated this problem in human lymphocyte cultures using various concentrations of drug and radiation.

MATERIALS AND METHODS

Peripheral blood lymphocytes were obtained from heparinized blood of six healthy volunteers by Urografin gradient centrifugation (47 ml aqua dest. plus 53.5 ml Urografin, sodium diatrizoate, 30%, adjusted to specific gravity 1085, Schering, Berlin, West Germany). 17–20 ml of blood were layered

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over 17 ml of Urografin and centrifuged at 1200 g for 30 min. The cells at the interface were removed and washed twice in medium 199 with Hanks' salts, 25 mM HEPES, L-glutamine (Gibco Bio-Cult, Glasgow, Scotland) at 300 g for 20 min. The washed cells were counted and resuspended in the above culture medium with 20% fetal calf serum, 100 u penicillin, and 100 µg of streptomycin/ml (Gibco Bio-Cult, Glasgow, Scotland). The final cell concentration was adjusted to approximately 1×10^6 cells/ml. The percentage of monocytes varied between 21 and 28% (α -naphthyl-acetate-staining).

8-MOP was taken from an alcoholic solution (Basotherm, Biberach a.d.Riss) and equal volumes of 8-MOP and dimethylsulphoxide (DMSO) were mixed in order to increase the solubility of the drug. The various concentrations used were obtained by diluting the solution with the above culture medium. The final concentration of ethanol and DMSO did not exceed 0.33% in any experiment and was found not to affect the stimulation capacity of the cultured lymphocytes.

The cells were distributed in 150 µl aliquots into flat bottom microtitration plates for culture (Greiner, Nürtingen, West Germany) and subsequently irradiated without sealing the plates. Phytohaemagglutinin P (Difco Laboratories Detroit, Michigan) 0.6 µg was added in 30 µl to each culture. In preliminary studies this mitogen concentration was found to give optimum blastogenesis. After incubation at 37°C for 3 days 0.37 µCi of ^3H -thymidine (^3H -TdR) (specific activity 5 Ci/mmol, Amersham, Buchler, Braunschweig, West-Germany) was added to each culture. The cells were harvested 4 h later on filter strips (Skatronas, Lierbyen, Norway) by means of a multiple cell-culture harvester (Titertek system, Flow Laboratories, Bonn, West-Germany).

Each filter strip was placed in 1 ml of Soluene 100 (Packard Instruments, GmbH, Frankfurt/M, West-Germany) at 60°C for 1 h and after this the vials were filled up with 5 ml of scintillation fluid (40 ml Permafluor R (Packard Instruments) plus Toluene at 1000 ml). Thymidine uptake was measured in a liquid scintillation counter. All experiments were done in quadruplicate.

The light source was a commercial blacklight unit (PUVA 4000, Waldmann, Schwenningen, West-Germany). The measured output was 4.5 mW/cm². Studies of cell viability were performed with trypan blue (0.5 g in 100 ml NaCl 0.9%).

Cell counts were performed on the Coulter counter Model Z.F. (Coulter Electronics, Dunstable, Beds. England). Cultures without PHA were studied on days 3 and 5 by dye exclusion and leukocyte counting. PHA stimulated cultures were examined by dye exclusion on day 3 only.

RESULTS

Using a standardized assay for the studies of lymphocyte transformation (Schlaak, 1975) highly reproducible incorporation rates of ^3H -TdR after treatment were obtained. Irradiation with UVA up to 3 J/cm² remained without a significant effect upon PHA-induced DNA synthesis (Fig. 1). Pre-incubation with 8-MOP at a concentration of 0.01 µg/ml inhibited the incorporation of ^3H -TdR slightly but significantly at 3 J/cm². However, when 8-MOP at concentrations of 0.1 and 0.5 µg/ml were added, a marked inhibition was obtained even at very low doses of UVA (Fig. 1). A more than 50% inhibition was obtained by using 0.1 µg 8-MOP per ml and 1 J/cm². Irradiation with 3 J/cm² and pre-incubation with 0.1 and 0.5 µg/ml 8-MOP produced complete unresponsiveness of the cells to PHA.

Cell viability was not affected by 8-MOP alone or by UVA irradiation alone up to 2 J/cm². While PHA stimulated lymphocytes remained viable after combined treatment with low energies (0.5 J/cm², Table 1), non-stimulated cultures showed an increasing number of trypan blue-positive cells. Treatment with high UVA (2 J/cm²) in the presence of 0.5 µg 8-MOP revealed a reduction of viable cells to approx. 50% when stimulated by PHA. In non-stimulated cultures cell death was less marked

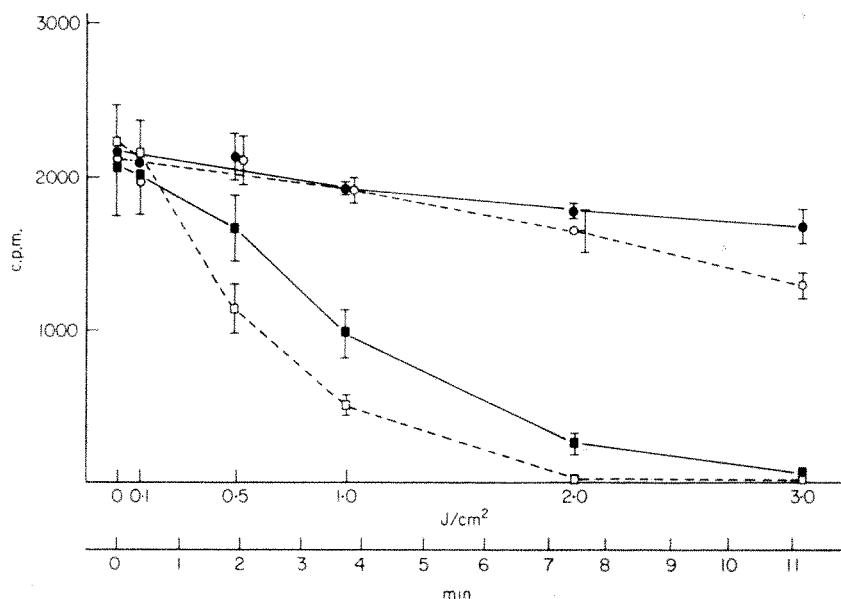


FIGURE 1. ^3H -thymidine incorporation in PHA stimulated human lymphocytes from 6 volunteers. Prior to PHA stimulation, the cells were incubated with various concentrations of 8-MOP and subsequently irradiated with UVA. Bars represent s.d. of the means. (●) Cells without 8-MOP; (○) 8-MOP 0.01 $\mu\text{g/ml}$; (■) 8-MOP 0.1 $\mu\text{g/ml}$; (□) 8-MOP 0.5 $\mu\text{g/ml}$.

TABLE 1. Viability of human peripheral lymphocytes after treatment with 8-MOP and UVA*

8-MOP ($\mu\text{g/ml}$)	UVA (J/cm^2)	Stimulated by PHA (3rd day)	Non-stimulated (3rd day)	Non-stimulated (5th day)
0.5	—	99.6 $\pm$ 5.0	94.6 $\pm$ 0.8	89.1 $\pm$ 4.4
—	0.5	98.0 $\pm$ 0.85	n.d.	n.d.
—	2	95.9 $\pm$ 4.9	94.4 $\pm$ 3.9	86.2 $\pm$ 3.2
0.5	0.5	95.5 $\pm$ 4.0	83.6 $\pm$ 0.9	54.6 $\pm$ 7.8
0.5	2	49.1 $\pm$ 8.2	76.1 $\pm$ 6.8	12.1 $\pm$ 8.1
Control (t.b. positive cells in %)			3.5 $\pm$ 1.7	11.1 $\pm$ 2.0

* Calculated as percent of control ($\pm$ s.d.).

after 3 days of combined treatment whereas after 5 days nearly 90% of the lymphocytes were found non-viable (Table 1).

DISCUSSION

The clinical effectiveness of oral 8-MOP followed by long wave ultraviolet irradiation has been well documented (Parrish *et al.*, 1974; Wolff *et al.*, 1976; Hofmann *et al.*, 1977). This therapeutic approach makes it necessary to achieve proper tissue levels of 8-MOP in the skin as well as high energy UV light sources, which sufficiently penetrate the skin in time. Little information is available concerning

the mode of action in diseased skin. Next to psoriasis, PUVA has been shown to be effective in treating subepidermal inflammatory diseases such as premycotic mycosis fungoides (Gilchrest *et al.*, 1976; Hofmann *et al.*, 1977). Therefore, lymphocyte cultures for investigating dose-relationships of 8-MOP and UVA were performed.

Our results show that PHA-induced DNA synthesis can be profoundly inhibited by pretreatment with low doses of 8-MOP and UVA. The minimum concentration of 8-MOP is found to be 0.1 µg 8-MOP/ml. Further reduction of the psoralen concentration to 0.01 µg/ml showed no effect. On the other hand, a 5 times increased concentration of psoralen (0.5 µg/ml) revealed only a moderately increased inhibitory activity as compared to 0.1 µg/ml (Fig. 1). Therefore, the minimal effective dose of 8-MOP in this cell system is around 0.1 µg/ml. Similarly, irradiation effects were strongly dose-dependent showing the smallest effective dose of UVA to be 0.5 J/cm². In most of the untreated patients an exposure dose of 1 J/cm² can be safely applied, so that energies in the range of 0.5 J/cm² are absorbed in the papillary layer. Therefore, the doses necessary partially to suppress the stimulatory effects of PHA upon DNA synthesis resemble those needed to obtain therapeutic results *in vivo*.

While the responsiveness of the cells to PHA was clearly reduced with 0.5 µg + 0.5 J/cm² as shown above, cell viability appeared not to be affected (Table 1). However, when 2 J/cm² were given, nearly half of the cells became non-viable. Even more pronounced was the effect in non-stimulated cells not undergoing a significant divisional activity. After 5 days only 10% of the cells remained viable. Interestingly, a similar dose of 8-MOP and UVA was found effectively to suppress human neutrophil chemotaxis *in vitro* (Langner & Christophers, 1977), which may not be connected with interstrand cross linking of DNA of replicating cells.

REFERENCES

- GILCHREST, B.A., PARRISH, J.A., TANENBAUM, L., HAYNES, H.A. & FITZPATRICK, T.B. (1976) Oral methoxsalen photochemotherapy of mycosis fungoides. *Cancer (Philadelphia)*, **38**, 683.
- GLINSKI, W., HAFTEK, M., OBALAK, S. & JABLONSKA, S. (1977) Clinical aspects of T and B lymphocytes in psoriasis. *Archives for Dermatological Research*, **258**, 89.
- HOFMANN, E., PLEWIG, G. & BRAUN-FALCO, O. (1976) Technische Erfahrungen mit der 8-Methoxypsoralen-Photochemotherapie bei Psoriasis vulgaris. *Hautarzt*, **27**, 277.
- HOFMANN, C., BURG, G., PLEWIG, G. & BRAUN-FALCO, O. (1977) Photochemotherapie kutaner Lymphome. *Deutsche Medizinische Wochenschrift*, **102**, 675.
- LANGNER, A. & CHRISTOPHERS, E. (1977) Leukocyte chemotaxis after *in vitro* treatment with 8-methoxypsoralen and UVA. *Archives for Dermatological Research*, **260**, 51.
- ORTONNE, J.P., CLAUDY, A.L., ALARIO, A. & THIVOLET, J. (1977) Decreased circulating E rosette forming cells in psoralen UVA treated patients. *Archives for Dermatological Research*, **258**, 305.
- PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, L. & PATHAK, M.A. (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. *New England Journal of Medicine*, **291**, 1207.
- SCHERER, R., KERN, B. & BRAUN-FALCO, O. (1977) The human peripheral lymphocyte—a model system for studying the combined effect of psoralen plus black light. *Klinische Wochenschrift*, **55**, 137.
- SCHLAACK, M. (1975) Zur Standardisierung und diagnostischen Bedeutung der phytomitogen-induzierten Lymphozytentransformation. *Immunität und Infektion*, **3**, 136.
- SWANBECK, G., THYRESSON-MÖK, M., BREDBERG, A. & LAMBERT, B. (1975) Treatment of psoriasis with oral psoralens and longwave ultraviolet light. *Acta dermato-venereologica*, **55**, 367.
- WOLFF, K., FITZPATRICK, T.B., PARRISH, J.A., GSCHNAIT, F., GILCHREST, B., HÖNIGSMANN, H., PATHAK, M.A. & TANENBAUM, L. (1976) Photochemotherapy for psoriasis with orally administered methoxsalen. *Archives of Dermatology*, **112**, 943.

Enhancement of comedogenic substances by ultraviolet radiation

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SUMMARY

Ultraviolet radiation enhanced the capacity of human sebum, sulphur, cocoa butter, squalene, and coal tar to produce comedones in the external ear canals of rabbits. An enhancement of the comedogenicity of coal tar and squalene was similarly demonstrated in man.

We conjecture that in occasional patients sunbathing may aggravate acne by augmenting the comedogenicity of sebum.

Everyone knows that sunlight is good for acne. Yet in a previous study Mills & Kligman (1978) were unable to demonstrate appreciably beneficial effects of various treatment regimens using ultraviolet radiation of different qualities. In addition, about one-fifth of the 207 patients interrogated about the effects of sunlight said they were made worse. The latter finding is in agreement with Cunliffe & Cotterill's (1975) survey of their acne population.

Jadassohn *et al*'s (1963) report that certain sunscreens are acneogenic made us wonder whether sunlight could abet the formation of comedones. We hypothesized that ultraviolet radiation might enhance the comedogenicity of sebum and of various exogenous substances.

We now report studies which show that ultraviolet possesses this property.

MATERIALS AND METHODS

Rabbit studies

Procedures for assessing the comedogenicity of chemicals in the external ear canals of albino rabbits have been described previously by Kligman & Mills (1972). The agent is applied daily for 5 weekdays for 2 weeks and the tissue sectioned horizontally on the 14th day for microscopic evaluation of follicular hyperkeratosis.

The test substances were: (1) pooled human sebum; (2) 2% elemental sulphur in Hydrophilic Ointment USP; (3) cocoa butter (neat); (4) 1% crude coal tar in petrolatum; (5) Noxzema (Noxell Co.) a cream commonly used to treat sunburn; and (6) squalene. All these are mildly to moderately comedogenic in the rabbit ear model.

Each substance was applied to opposite ears of 5 rabbits. On Mondays, Wednesdays and Fridays.

for 2 weeks, one ear canal received 2 MED's (individually determined beforehand) of UV radiation using a 1600 W solar simulator described by Berger (1969). Access of radiation to the canal was provided by surgically splitting the canals under anaesthesia. This made it possible to expose the deepest recesses. Five control rabbits received a non-comedogenic cream, Hydrophilic Ointment USP, and a similar schedule of radiation to one side. To rule out that inflammation itself could enhance comedogenicity four groups of five rabbits each received respectively Noxzema, cocoa butter and 1% crude coal tar to both ears; one side was frozen for 20 s thrice weekly for 2 weeks with ethyl chloride. A matching study utilized 10% aqueous phenol daily to one side to create a scaling, inflammatory reaction of moderate severity. Phenol neither induces nor reduces comedones in rabbit ear assays as shown by Mills & Kligman (1975).

Human studies

The test agents were applied under continuous occlusion for 4 weeks to the upper back, each to a separate group of five young, adult males with a prior history of acne. Three comedogenic substances were involved: 15% crude coal tar in Hydrophilic Ointment USP, Noxzema and squalene (neat). Hydrophilic ointment served as the control. The agents were liberally applied to 8 cm squares of skin which were then covered with identically sized squares of saran wrap. The preparation was held to the skin under overlapping strips of impermeable plastic tape. Fresh applications were made thrice weekly. On Mondays, Wednesdays and Fridays, just before renewing the applications, one-half of each square received three MED's of erythemic radiation from a bank of fluorescent sunlamps (Westinghouse FS20 lamps) continuous emission 280–320 nm followed by 15 min of UV-A radiation from a bank of fluorescent blacklights (Westinghouse, peak emission 360 nm). The physical characteristics of these light sources have been reviewed by the Task Force on Photobiology of the National Program for Dermatology (1974). The approximate dose of UV-A was 1.3 J/cm². This exposure produced an erythematous, scaling dermatitis which peaked by the end of four weeks accompanied by strong tanning.

On the Monday after the last exposure, tissue was excised by scalpel from both sides of each square, fixed in formalin, semiserially sectioned and stained with haematoxylin and eosin. The degree of comedo formation was assessed histologically on a 4-point scale: 0 = none; 1 = slight; 2 = moderate; and 3 = strong.

RESULTS

Rabbits

With each of the comedogenic agents, the addition of solar simulating radiation enhanced the degree of follicular hyperkeratosis (Table 1). The highest score, 3, does not really reflect the full impact of sunburning radiation on comedo formation. The much greater distension of follicles with horny material on the irradiated side was easily evident by hand-lens inspection of the sections on the slides. Hydrophilic ointment was not comedogenic and did not become so after irradiation. In none of the ears treated with ethyl chloride or 10% phenol was there any evidence that the induced inflammation had an augmenting effect on comedo formation. Follicular hyperkeratosis was not influenced in either direction.

Humans

Hydrophilic ointment did not induce comedones, with or without irradiation. In only one site of one subject were small, closed comedones grossly visible, this being with Noxzema on the irradiated side.

TABLE 1. Effect of irradiation on comedogenicity

Substances	Grades									
	Non-irradiated					Irradiated				
	1	2	3	4	5	1	2	3	4	5
Rabbit										
Cocoa butter	2	2	2	2	2	3	3	3	3	3
1% Coal tar	2	2	3	2	3	3	3	3	3	3
Human sebum	1	1	1	1	1	2	2	3	3	3
Noxzema	1	1	2	1	2	2	3	3	3	2
2% Sulphur	2	2	2	2	2	3	3	3	3	3
Squalene	2	2	1	2	1	3	3	2	3	2
Hydrophilic ointment	0	0	0	0	0	0	0	0	0	0
Human										
15% Coal tar	2	2	2	2	2	3	3	3	3	3
Noxzema	1	1	1	1	1	2	2	2	2	2
Squalene	1	2	1	1	1	2	2	2	1	2
Hydrophilic ointment	0	0	0	0	0	0	0	0	0	0

Histological study showed mild hyperkeratosis in all five unirradiated, Noxzema and squalene sites. In each case UV radiation sharply increased the size of the micro-comedones.

An admixture of open and closed comedones was clinically apparent in each of the five subjects receiving 15% coal tar without radiation. A sprinkling of follicular papules was also present. Comedones and papules became more numerous and larger when irradiated, an effect already evident by 2 weeks. The potentiation of comedo formation was even more marked in histological sections (Figs 1 and 2). It should be noted that the inflammatory reaction to 3 MED's in coal tar sites was greater than with Noxzema or squalene owing to the inherent phototoxicity of coal tar. This was also the case in the rabbit assays.

Although on a short-term basis we did not anticipate significant clinical changes with agents demonstrating mild or moderate comedogenicity, three subjects tested with squalene developed numerous papules and pustules in the irradiated sites 4-6 weeks after the last exposure. The control sites showed but a slight increase (Fig. 3). This finding underlines the importance of the possible effects of ultraviolet light's potentiation of comedogenicity in susceptible individuals.

DISCUSSION

These studies show that UV radiation similar to that of sunlight has a potentiating effect on comedogenic substances. We think it a possibility that daily sunbathing might enhance the response of the follicular epithelium to comedogenic substances native to the follicle, namely, sebum and comedogenic products of *P. acnes*. Thus, while the immediate effects of sunbathing (more rapid resolution of pustules) might seem beneficial to most patients, the long term result might well be to amplify the creation of new comedones, thereby perpetuating the disorder. This scenario would then be analogous to the one Mills & Kligman (1972) formulated regarding the effects of sulphur in acne. Mopping up inflammatory lesions after comedones have burst is not very important if in fact new 'time bombs' are being laid down all the time.



FIGURE 1. Horizontal section showing comedo formation from continuous exposure to 15% coal tar for 4 weeks (H & E, $\times 110$).

Some of our acne patients experience flares of acne so quickly when exposed to intense sunlight that some other mechanism besides intensified comedo formation has to be involved. Many acne patients have fragile follicles which may become easily damaged by chemical and physical agencies once hyperkeratosis has begun.

From these and other observations we hold that as an acne treatment the baleful effects (aging changes) of sunbathing greatly outweigh the benefits. We concede that papulo-pustules are more rapidly resorbed, but the formation of comedones is encouraged. Since it takes 3-4 months for comedones to become visible from the time of microscopic inception, the enhancement of comedogenicity is entirely sub rosa. Because the harmful effects are invisible and the beneficial effects on inflammatory lesions so easily perceived we think it unlikely that our arguments will carry much weight with the majority of patients and doctors who believe in the good effects of sunlight. We warn acne patients against excessive sunbathing and urge the daily use of effective sunscreens in any case.

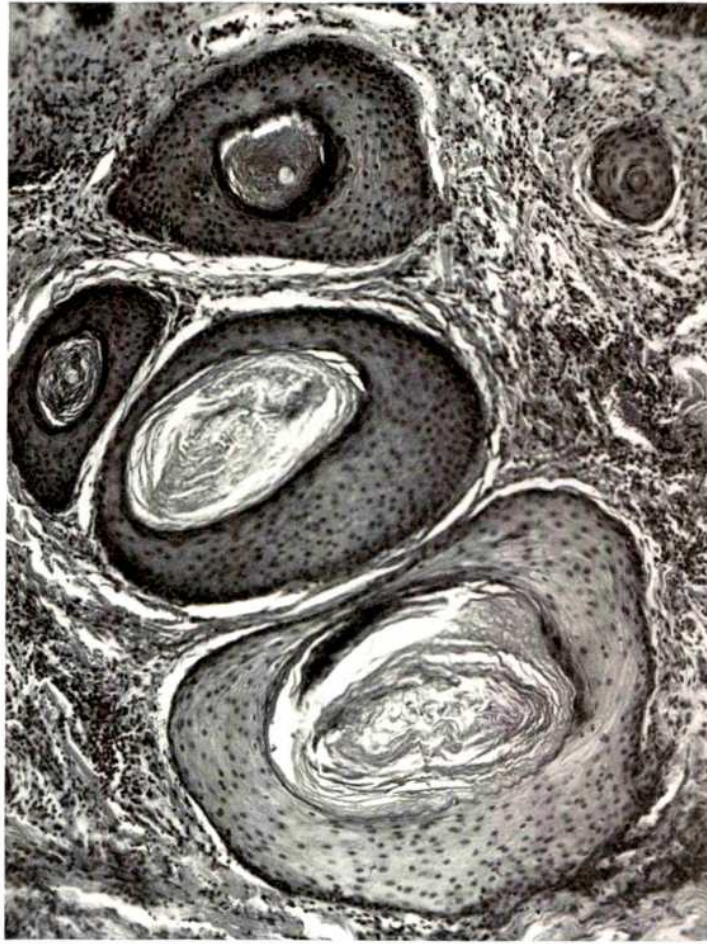


FIGURE 2. Enhancement of hyperkeratosis by radiation (H & E, $\times 110$).

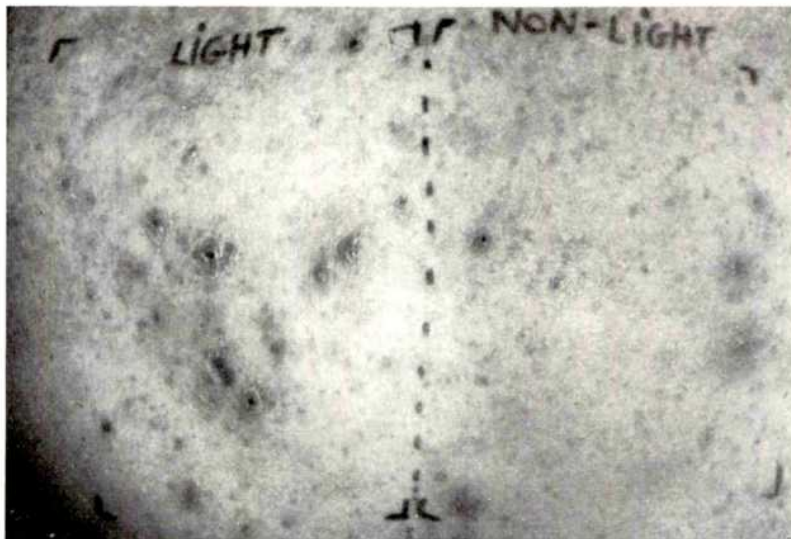


FIGURE 3. Four weeks post radiation. UV treated side contains numerous deep papules and pustules compared to squalene alone.

REFERENCES

- BERGER, D.S. (1969) Specification and design of solar ultraviolet solar simulators. *Journal of Investigative Dermatology*, **53**, 192.
- CUNLIFFE, W.J. & COTTERILL, J.A. (1975) *The Acnes*, p. 15. W.B. Saunders, London.
- JADASSOHN, W., PAILLARD, R., HUNZIKER, N., LOZERON, H. & MAGGIORA, A. (1963) Keratose follicular 'epidemie'. *Dermatologica*, **127**, 152.
- KLIGMAN, A.M. & MILLS, O.H. (1972) Acne cosmetica. *Archives of Dermatology*, **106**, 843.
- MILLS, O.H. & KLIGMAN, A.M. (1975) Assay of comedolytic agents in the rabbit ear. In: *Animal Models in Dermatology* (ed. by H.Maibach) pp. 176-183. Churchill Livingstone, New York.
- MILLS, O.H. & KLIGMAN, A.M. (1972). Is sulphur helpful or harmful in acne vulgaris? *British Journal of Dermatology*, **86**, 620.
- MILLS, O.H. & KLIGMAN, A.M. (1978) Ultraviolet phototherapy and photochemotherapy of acne vulgaris. *Archives of Dermatology*, in press.
- THE TASK FORCE OF PHOTOBIOLOGY OF THE NATIONAL PROGRAM FOR DERMATOLOGY (1974) Report on ultraviolet light sources. *Archives of Dermatology*, **109**, 833.

Psoriasis and stress. II

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SUMMARY

A retrospective assessment of insight was undertaken in 61 patients whose psoriasis was considered to have been precipitated by stress. A significantly better prognosis was observed in those patients assessed as having had insight.

It has been shown that psychological stress could precipitate psoriasis; the prognosis was then much better than when no prior stress had been recollected by the patient (Seville, 1977). The incubation time from stress to rash, as remembered by the patient, was between 2 days and 4 weeks; two-thirds of the instances occurred within a fortnight. Clinically it was observed that the better prognosis followed the development of insight by the patient, that is awareness and acceptance of the precipitating factors. Proof of this observation was therefore attempted.

MATERIALS AND METHOD

132 patients were completely cleared with dithranol and carefully followed up for 3 years as has been previously described (Seville, 1977). No local steroids were used. Only first attacks of psoriasis were assessed. Fifty-one patients had recollected specific incidents of stress within 1 month prior to the onset of their first attack of psoriasis. Another 10 patients had experienced specific incidents of stress of similar intensity but were not quite as certain of the time relationship of stress and rash. The incidents were found to fall under the same headings and in the same proportions as previously described, major upsets within the family group being the most frequent. Their case histories were checked by an independent medical observer (G.B.W.) who agreed that the stress had been specific and of similar degree as experienced by the 51 patients.

Patients who recalled stress were initially given a lay explanation of how the stress of an upset could be retained subconsciously in the mind, like a Pavlov reflex, with perpetuation of the eruption; and after recollection, providing there was insight, there could be release of the repressed stress.

A retrospective assessment of insight in the 61 patients was then attempted at follow up for a period of 3 years, a proforma being completed under the following headings: whether the explanation had been understood by the patient and fundamentally accepted, indicating that there had been insight; or whether the explanation had been rejected, forgotten, or not understood, suggesting the absence

of insight. Answers in the affirmative to any of the three latter headings would occur in those unwilling or unable to accept the relevance of the upset.

RESULTS

There is a significantly better prognosis where insight is present, as can be seen as follows:

		Clear at $\frac{1}{4}$ year	Clear at 1 year	Clear at 3 years
Insight	31	31 (100%)	29 (93%)	23 (74%)
No insight	30	14 (47%)	10 (33%)	6 (20%)
Significance		$4.7 \times \text{s.e.}$ $P < 0.001$	$4.9 \times \text{s.e.}$ $P < 0.001$	$4.2 \times \text{s.e.}$ $P < 0.001$

Severely affected patients were not excluded as previously (Seville, 1977), as 5 were present in each group.

When the patients with no insight are compared under the three following headings, it can be seen that there has been a varying lack of acceptance of either the explanation or the reality of the upset:

	No insight	30	Clear at $\frac{1}{4}$ year	Clear at 1 year	Clear at 3 years
Not understood	14		7 (50%)	5 (36%)	4 (29%)
Forgotten	10		5 (50%)	4 (40%)	2 (20%)
Rejected	6		2 (35%)	1 (17%)	0 (0%)

None of the patients classified under the heading 'forgotten' was over 40 and this therefore tends to exclude forgetfulness due to age.

There was no significant difference in the tendency to psoriasis as judged by the presence of a positive family history, between those in the previous article (1977) where stress had not been recollected and in the present series, whether there was insight or no insight.

DISCUSSION

Insight in this article has been defined as self-knowledge: that is observation by the patient of the facts of what has happened, resulting in an understanding in depth with full acceptance; not introspection, nor the veneer of superficial recognition which dodges real awareness. Consultation between doctor and patient is always individual, but it was thought that an accurate assessment could be made from the proforma headings already mentioned, in conjunction with any nuances or reactions by the patient when questioned at follow up.

It could be argued that patients who relapsed might reject or even forget the explanation and therefore be assessed as not having insight. Even if this were so, only a small number rejected which did not affect significance. Those who relapsed more quickly were assessed earlier with less time for

forgetting. A full scale prospective assessment and with greater numbers is needed for absolute proof of the importance of insight.

Indirect but prospective evidence is available from the proformas; hardly any additional history was obtained at follow up in those who did well, whereas considerable extra detail in depth was discovered in those who did badly.

There was no significant difference in the rate of relapse between patients described in the previous article where stress had not been recollected, and those in this series assessed as having had no insight. Patients having insight had a significantly better prognosis.

The amount of time spent on all of the patients at repeated consultations to achieve these results is hardly ever available at busy ward rounds and clinics. How much more fundamental would it be for the history and its explanation to be carried out by the family practitioner right at the onset of the rash? Memory would then be recent and recollection more readily encouraged for the background would probably be known.

The continuing repression of an upset is likely to be followed by chronicity. Complete clearance with physical treatment is usually necessary to improve the prognosis. An even more lasting effect may follow early therapy not only to the skin but to the patient as a whole.

REFERENCE

SEVILLE, R.H. (1977) Psoriasis and stress. *British Journal of Dermatology* **97**, 297.

Addendum

In the paper 'Psoriasis and stress (I)' by R. H. Seville (*British Journal of Dermatology*, 1977, **97**, 297), the percentage cure rates were unfortunately transposed. The correct version of the figure and legend is as follows:

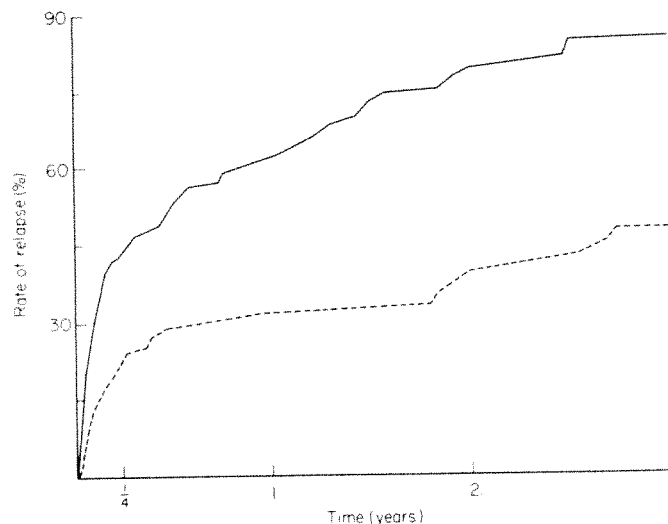


FIGURE 1. Relapses of 51 patients with prior recollected stress (---) (53% clear after 3 years), 81 where none had been recalled (—) (17% clear after 3 years).

Immunofluorescent tracing of cytoplasmic components involved in keratinocyte differentiation

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SUMMARY

Sera with epidermal cytoplasmic antibodies (E.C.A.) produced by human subjects after bone marrow transplantation have been studied by indirect immunofluorescence on rabbit lip sections. These sections were used in order to give on a single specimen, areas of mucosa, of parakeratotic and of orthokeratotic epithelium. It has been demonstrated that E.C.A. distinguished the basal cell layer from the upper cell layers on the three areas. Moreover, E.C.A. were not found to react identically on mucosal, parakeratotic and orthokeratotic epithelium. These findings strongly suggest that the target antigens for E.C.A. are molecules involved in keratinocyte differentiation.

We have previously found that patients undergoing bone marrow transplantation frequently produce immunoglobulins G which react by indirect immunofluorescence (I.I.F.) with the cytoplasm of epidermal cells (Saurat *et al.*, 1976). Sera with epidermal cytoplasmic antibodies (E.C.A.) were found by I.I.F. to react either with the cytoplasm of the basal cell layer or with the cytoplasm of the 'upper' cell layers (which included spinous, granular and horny layers) (Saurat *et al.*, 1977). Such a distinct distribution of cytoplasmic staining by I.I.F. on epidermal substrates has also been observed by other investigators (Bystryń *et al.*, 1973; Burnham 1974; Ackerman Schopf *et al.*, 1974). The hypothesis that the localization of the staining to the basal or to the upper cell layers could be related to the type of substrate has not been previously considered. In a preliminary study we found that the distribution was distinct when a serum with E.C.A. was concomitantly studied on human skin and on rabbit oesophagus (Saurat *et al.*, 1977); since the difference could be due to the fact that the former was a keratinized substrate and the latter a mucosal one, we have studied the distribution of the staining on one single substrate having both a mucous side and a keratinized side. We describe in this report the results of these experiments which suggest that substances involved in keratinocyte maturation could be the target antigens for E.C.A. and therefore that E.C.A. could be used for the study of keratinocyte differentiation.

This work has been presented in part at the meeting of the European Society for Dermatological Research, Amsterdam 2-4 May 1977.

MATERIALS AND METHODS

Sera

These were obtained from patients undergoing bone marrow transplantation. They have been assessed for E.C.A. content by indirect immunofluorescence on rabbit oesophagus and on human skin as previously described (Saurat *et al.*, 1976, 1977). Seven sera from seven subjects were selected; their characteristics are given in Table 1. Sera were used diluted 1/10, 1/50 and 1/100.

Substrates

Specimens were snap frozen in liquid nitrogen; rabbit lip and oesophagus as well as group O human skin were used. Four μ m cryostat (-25°C) sections of each substrate were applied on each slide. The rabbit lip was obtained from the junctional zone between the skin side and the mucosal side; such a section gives on a single specimen areas of mucosa, of parakeratotic and of orthokeratotic epithelium (Fig. 1). Sections were controlled by routine histological examination. Ten rabbits were used.

Labelled antisera

Commercially available (Behring) fluorescein isothiocyanate (F.I.T.C.) labelled rabbit anti-human IgG was used and had the following properties: antibody concentration 1 mg/ml; F.I.T.C. to protein ratio 2.3 mol; working dilution 1/10.

Technique

Standard indirect immunofluorescent techniques were used. Briefly: cryostat sections were incubated for 30 min with the serum, washed for 30 min in PBS, treated with the conjugate for 30 min, washed again for 1 h and mounted in buffered glycerol. A Leitz Orthoplan microscope with vertical illuminator for epi-illumination was used with an HBO 200 mercury vapour light and filters comb. I and S525 Leitz.

Controls

See also Table 4. The labelled antiserum was studied each time on the substrate in a one step method. Human sera (diluted 1/10) lacking E.C.A. were used as negative controls in each experiment. Two

TABLE 1. Characteristics of the sera with epidermal cytoplasmic antibodies (E.C.A.) used in this study

Serum no.	1	2	3	4	5	6	7
Rabbit oesophagus							
No. of substrates used	6	6	42	5	4	6	5
Type of staining	B.E.C.	B.E.C.	B.E.C.	B.E.C.	B.E.C.	G.E.C.	G.E.C.
Titre	200	10	100	100	50	200	200
Ig class	G	G	G	G	G	G	G
Human skin							
No. of substrates	42	46	31	34	6	40	6
Type of staining	B.E.C.	U.E.C.	U.E.C.	U.E.C.	U.E.C.	B.E.C.	Negative*
Titre	200	10	100	50	50	200	—
Ig class	G	G	G	G	G	G	—

* Staining of the basal cells of the outer root sheath of the hairs. B.E.C. (basal epidermal cytoplasm) staining of the basal cells. U.E.C. (upper epidermal cytoplasm) staining of the spinous, granular and horny cells. G.E.C. (general epidermal cytoplasm) staining of all the layers. Data from Saurat *et al.*, 1976, 1977.

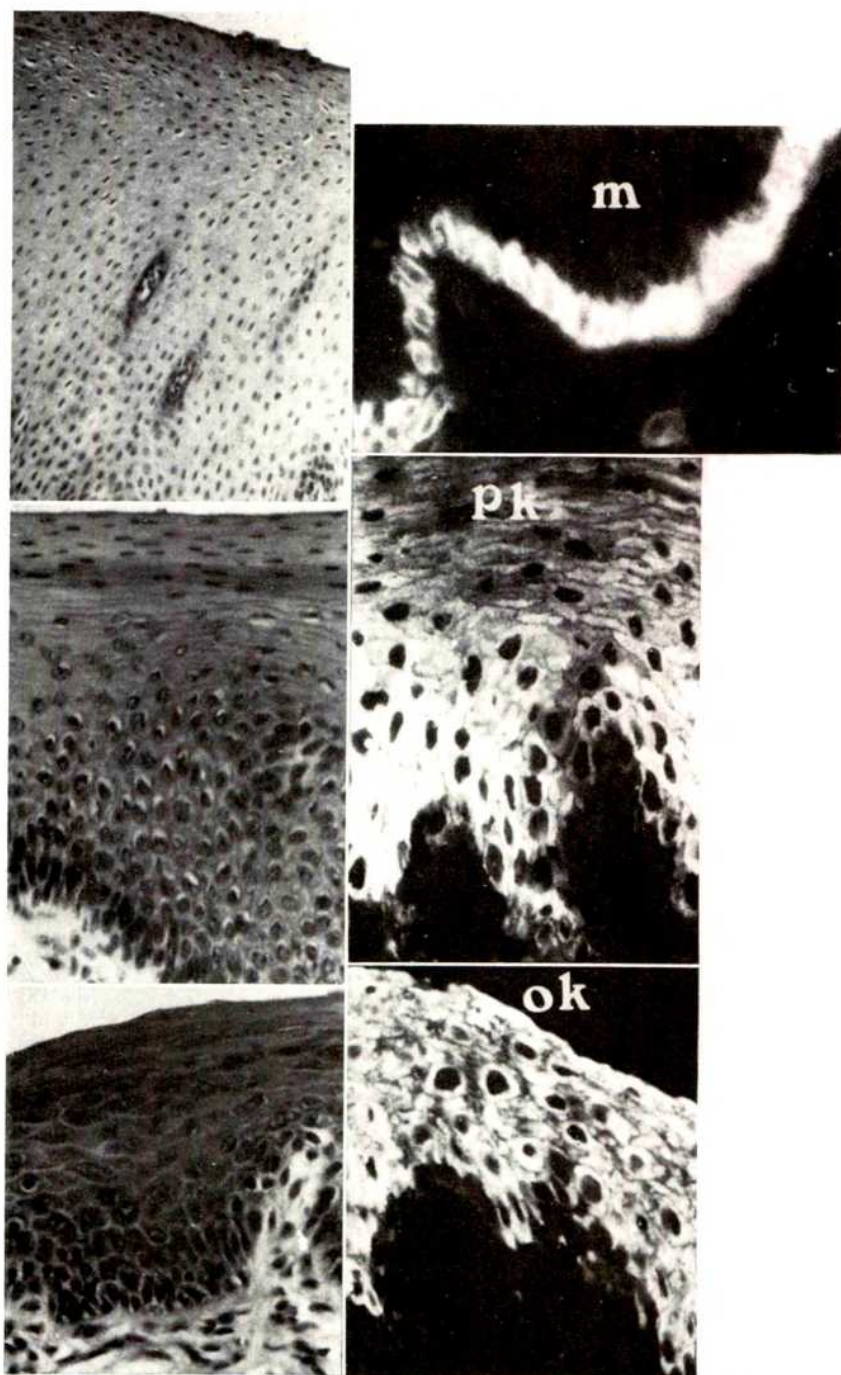


FIGURE 1. The distribution of the staining pattern obtained with a single serum (Serum no. 3 1/50; Tables 1 and 2) by indirect immunofluorescence on a single rabbit lip specimen: there is staining of the basal cells on the mucous side (m, histology on the left); on the parakeratotic (P.K.) and orthokeratotic (O.K.) sides there is staining of the upper compartment; the basal cell layer is not stained. (F.I.T.C. labelled anti IgG. Epi-illumination. Objective 40×0.65 . Ektachrome high speed 160 ASA).

kinds of blocking tests were done as follows: (i) the F.I.T.C. labelled anti IgG was incubated for 1 h at 37°C and for 24 h at 4°C with purified human IgG (kindly provided by Dr Preud-Homme) in antigen excess: (F.I.T.C. anti IgG 1 mg/ml, IgG 7 mg/ml, or 3.5 mg/ml). The solutions were centrifuged at 5000 rev/min for 1 h and the supernatant was used as test reagent in I.I.F. The same procedure was also used as control with purified human IgM. (ii) Standard blocking test: the specimens were initially incubated with the serum containing E.C.A.; unlabelled rabbit anti human IgG was applied (antibody content 1.23 mg/ml) or, as control, a non immune rabbit serum; the F.I.T.C. anti human IgG was then used. The binding of free F.I.T.C. on the substrates was also studied as follows: free F.I.T.C. (0.25 µg/ml in H₂O) was applied to the substrate for 2 min; the specimen was then washed twice (2 min) in water and incubated in acetic acid according to Christophers & Braun Falco (1971). As this procedure was not closely similar to the conditions of I.I.F., free F.I.T.C. (1 µg/ml in PBS) was also applied (30 min) on the substrate previously incubated (30 min) with a serum containing E.C.A.; the slides were then washed for 30 min in PBS without use of acetic acid.

TABLE 2. Distribution of the cytoplasmic staining on different substrates

Serum no.		Rabbit lip						Human skin (orthokeratotic)
		Rabbit oesophagus (mucous)	Mucous side		Parakeratotic side		Orthokeratotic (Interfollicular) side	
1	U	—	—	—	—	—	—	—
	B	(10)	(10)	(10)	(10)	(10)	(10)	(10)
2	U	—	—	—	—	—	—	—
	B	(1) (8)	(1) (8)	(1) (8)	(1) (8)	(9)	(9)	(9)
3	U	—	—	—	—	—	—	—
	B	(10)	(10)	(10)	(10)	(10)	(10)	(10)
4	U	—	—	—	—	—	—	—
	B	(7)	(1) (7)	(1) (7)	(1) (7)	(8)	(8)	(8)
5	U	—	—	—	—	—	—	—
	B	(1) (7)	(3) (7)	(3) (7)	(3) (7)	(10)	(10)	(10)
6	U	—	—	—	—	—	—	—
	B	(9)	(1) (8)	(1) (8)	(1) (8)	(9)	(9)	(9)
7	U	—	—	—	—	—	—	—
	B	(2) (6)	(2) (6)	(2) (6)	(2) (6)	(8)	(8)	(8)
		+	+	+	+	—(+)*	—(+)*	

* Staining of the basal cells of the outer root sheath of the hairs.

U = upper; B = basal (see Table 1 and Fig. 1).

Figures in parentheses = numbers of experiments.

+, Cytoplasmic staining; —, no cytoplasmic staining.

RESULTS

Distribution of the staining

Table 2 shows that: (i) two compartments could be distinguished: the basal cell layer and the supra-basal cell layers or 'upper compartment' (Fig. 1). (ii) The staining of one compartment instead of the other one was related to the type of substrate; sera were found to react only with the basal cell compartment on the mucous side of the substrate and only with the upper compartment on the orthokeratotic side of the substrate and vice versa. However, staining of the upper compartment was never seen alone on the mucous side. (iii) There were junctional zones in which the distribution of the staining changed: these were either at the junction between the mucous and the parakeratotic zones (Fig. 2) or at the junction between the parakeratotic and the orthokeratotic areas. The distinct

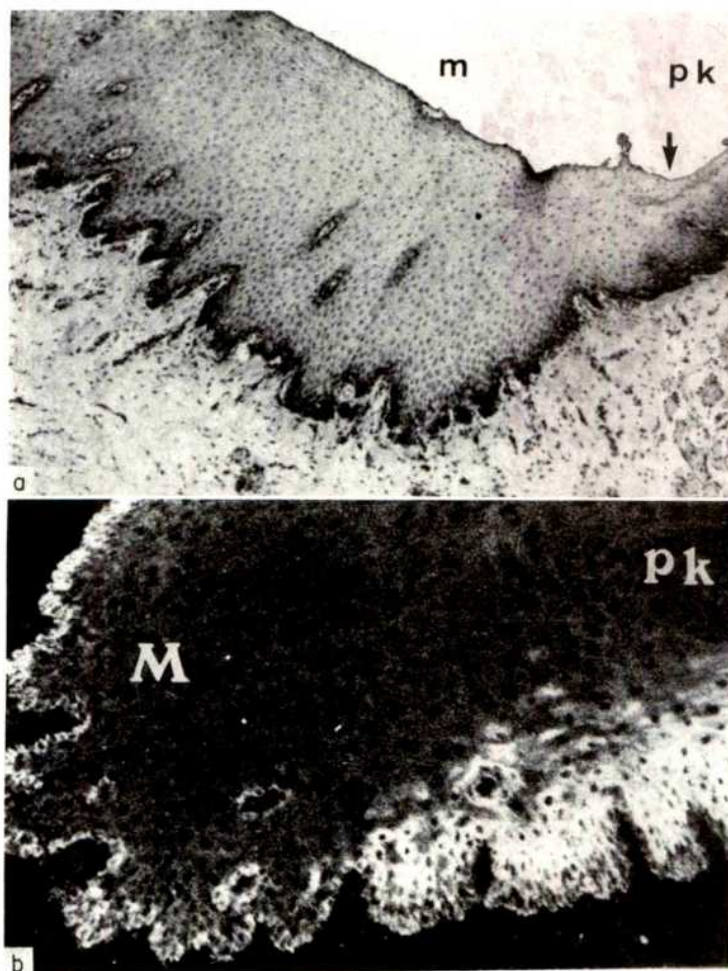


FIGURE 2. (a) Junctional zone, on a rabbit lip specimen, between mucous side (M) and parakeratotic side (P.K.). (b) Distribution of the staining pattern observed by I.I.F. with a single serum (no. 3 see Tables 1 & 2 and Fig. 1). It can be seen that the distribution of the staining changes in this junctional zone: there is a basal staining on the mucous side and a progressive upper staining as the parakeratosis begins.

distribution of the staining was most probably due to changes in the antigenic properties of the substrate as it passed from mucosa to parakeratinization and then to orthokeratinization: Table 3 shows that each of these changes was recognized by several sera. It has not been directly established whether each serum reacted with several components (i.e. was a mixture of antibodies with distinct specificities) or a single serum reacted with one single component. The former hypothesis would be best correlated with our results (Table 3).

Controls for specificity

(i) The distinct staining distribution was not an artifact due to the sectioning of the substrates because it was very reproducible; the same staining was obtained with one serum on several sections from a single substrate as well as on substrates from several animals (see Table 2). Moreover sera containing pemphigus antibodies were found to react uniformly on the different parts of the substrates. These observations are further supported by the results obtained in an even larger series using a comparison between oesophagus and skin (Table 1). (ii) The staining obtained with the E.C.A. was not a 'non specific staining' (Table 4). Free F.I.T.C. alone gave a different staining of the substrate; according to Christophers & Braun Falco (1971) there was a faint cytoplasmic staining but without the distinct

TABLE 3. The different staining patterns observed with a single serum all along the rabbit lip (Table 2) were most probably related to changes in the reactivity (i.e. antigenic properties) of the substrate as it passed from mucous differentiation to parakeratotic or orthokeratotic differentiation

(3a)	Basal staining (B)			Upper staining (U)		
	Mucous	Para- keratotic	Ortho- keratotic	Mucous	Para- keratotic	Ortho- keratotic
	BI	BII	BIII	UI	UII	UIII
Serum no.						
1	+	+	+	—	—	—
2	+	+	—	—	+	+
3	+	—	—	—	+	+
4	—(+)	—	—	—	—(+)	+
5	—(+)	—	—	—	—(+)	+
6	+	+	+	+	+	—
7	+	+	—	+	+	—

(3b)	Differences between	Shown by serum no.
	BI—BII	3. (4). (5)
	BI—BIII	2.3.7. (4). (5)
	BII—BIII	2.7.
	UI—UII	2.3. (4). (5)
	UI—UIII	2.3.4.5.6.7.
	UII—UIII	6.7. (4) (5)

B = basal; U = upper (see Table 2).

Signs and figures in parentheses represent the results of a small number of positive experiments which differed from a larger number of experiments done with the same serum (see Table 2).

TABLE 4. The cytoplasmic staining (Figs 1 and 2) obtained on rabbit lip sections was not non specific. Properties of the antisera used have been given previously (Saurat *et al.*, 1976)

(1) Incubation of the substrate with		Human sera with epidermal cytoplasmic antibodies (E.C.A.)										Normal human sera		Nothing			
(2) Staining with																	
Cyto- plasmic stain- ing*	++ +	F.I.T.C. anti human IgG		Unlabelled rabbit serum anti human IgG		Non immune rabbit serum		Unlabelled rabbit anti guinea-pig Ig serum		F.I.T.C. anti human		IgM	IgA	IgE	C3	IgG	IgG
	++ +	Absorbed with purified IgG		Absorbed with purified human IgM		F.I.T.C. anti human IgG											
	++ +			±		++ +		++ +				-	-	-	-	-	-
	++ +																
	++ +																

distribution which was found in I.I.F. with E.C.A. Neither did incubation of the substrate with E.C.A. followed by addition of free F.I.T.C. result in a distinct distribution of the staining; this fact ruled out a possible interaction of F.I.T.C. with a substance present in sera with E.C.A. which would have bound to the substrates and then fixed free F.I.T.C.

DISCUSSION

These findings demonstrate that the localization of the cytoplasmic staining either to the basal cell layer only or to the 'upper' cell layers only may be related to the nature of the substrate. From a practical point of view it would thus appear necessary to characterize each serum with E.C.A. by I.I.F. on a keratinized and on a mucous substrate. Theoretical implications of these findings are two-fold:

- (i) E.C.A. distinguish the basal cell layer from the upper cell layers.
- (ii) E.C.A. distinguish the keratinized from the non-keratinized Malpighian epithelia.

(i) *Distinction between basal and upper compartments*

This distinction is obvious in this work. It is supported by the fact that several workers have reported 'basal cell antibodies' (Thivolet & Beyvin, 1968; Van Joost, 1974; Ackerman Schopf *et al.*, 1974; Bystryń, 1976; Ackerman Schopf, 1976; Ablin, 1976; Shmunes & Shu, 1977) or 'upper' cell antibodies (Bystryń *et al.*, 1973; Burnham, 1974; Ogawa *et al.*, 1975; Abel & Bystryń, 1976).

This means that each compartment has, in its cytoplasm, components which differ in their antigenic properties (as far as we can assume from an immunofluorescent picture that the target antigens are actually in the cytoplasm). Such an abrupt change between the basal cell layer and the layers just above is puzzling; most of the histochemical methods do not show such an abrupt distinction. Moreover these observations are not in accordance with the concept of the basal cells being composed of several subpopulations (reviewed by Christophers & Laurence, 1976); however, from evidence obtained by electron microscopy the cytoplasm of the basal cells shows differences as compared to the cells which are just above them (Breathnach, 1971). Such an abrupt change would be in accordance with a concept that the basal cell is uncommitted and that the cell just above has entered the cycle of differentiation (committed). This 'commitment' should have a molecular expression and E.C.A. could be markers for some of these molecules.

(ii) *Distinction between mucous, parakeratinized and orthokeratinized epithelia*

The sera did not react identically all along the entire rabbit lip sections made through the junctional mucous—parakeratotic—orthokeratotic areas. There is strong evidence that this was not an artifact. These findings most probably reflect differences in some cytoplasmic structures depending on whether the cell is involved in a mucous, a parakeratinized or an orthokeratinized differentiation. To our knowledge, markers for such differentiation components have not been available so far. It is likely that E.C.A. could react with such components. In conclusion, further work seems to be justified to establish the localization within the cell as well as the molecular nature of the targets of E.C.A. which could then be used as markers for the keratinocyte differentiation process.

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REFERENCES

- ABEL, E.A. & BYSTRYN, J.C. (1976) Epidermal cytoplasmic antibodies: incidence and type in normal persons and patients with melanoma. *Journal of Investigative Dermatology*, **66**, 44.
- ABLIN, R.J. (1976) Immunologic sequelae of thermal injury. I. Frequency and relationship of epithelial antibodies to extent of burn. *Clinical Immunology and Immunopathology*, **5**, 195.
- ACKERMAN SCHOPF, C., ACKERMAN, R., TERAZAKI, P.I. & LEVY, J. (1974) Natural and acquired epidermal auto antibodies in man. *Journal of Immunology*, **112**, 2063.
- ACKERMAN SCHOPF, C. (1976) Antibodies to basal epidermal cells in patients with basal or squamous cell carcinoma. *Archives of Dermatological Research*, **257**, 149.
- BREATHNACH, A.S. (1971) In: *An Atlas of the Ultra structure of Human Skin*, p. 114. Churchill Livingstone, London.
- BYSTRYN, J.C., ABEL, E. & WEIDMAN, A. (1973) Antibodies against the cytoplasm of human epidermal cells. *Archives of Dermatology*, **108**, 241.
- BYSTRYN, J.C. (1976) Clinical significance of basal cells antibodies. *Clinical Research*, **24**, 262.
- BURNHAM, T.K. (1974) Two epidermal cytoplasmic immunofluorescent patterns detected by indirect immunofluorescence. *Journal of Investigative Dermatology*, **62**, 100.
- CHRISTOPHERS, E. & BRAUN FALCO, O. (1971) Fluorescein isothiocyanate as a stain for keratinizing epithelium. *Archiv für Dermatologische Forschung*, **241**, 199.
- CHRISTOPHERS, E. & LAURENCE, E.B. (1976) Kinetics and structural organisation of the epidermis. In: *Current Problems in Dermatology* (Ed. by J.W.H. Mali), Vol. 6, p. 87. Karger, Basel.
- OGAWA, H., MIYAZAKI, I., IKEYA, T., MASUTANI, M., TANEDA, A. & SANADA, A. (1975) Presence of anticytoplasmic antibodies in a case of incontinentia pigmenti. *Journal of Dermatology*, **2**, 93.
- SAURAT, J.H., BONNETBLANC, J.M., GLUCKMAN, E., DIDIERJEAN, L., BUSSEL, A. & PUISSANT, A. (1976) Skin antibodies in bone marrow transplanted patients. *Clinical and Experimental Dermatology*, **1**, 377.
- SAURAT, J.H., GLUCKMAN, E., DIDIERJEAN, L., SOCKEEL, F., BONNETBLANC, J.M. & PUISSANT, A. (1977) Anticorps anti cytoplasme des cellules épidermiques humaines. *Annales de Dermatologie et Vénérologie*, **104**, 121.
- SHMUNES, E. & SHU, S. (1977) Bullous disease of childhood—report of a case demonstrating anti basal cell antibody. *Archives of Dermatology*, **113**, 325.
- THIVOLET, J. & BEYVIN, A.J. (1968) Recherche par immunofluorescence d'auto anticorps sériques vis à vis des constituants de l'épiderme chez les brûlés. *Experientia*, **24**, 945.
- VAN JOOST, T. (1974) Incidence of circulating antibodies reactive with basal cells of skin in drug reaction. *Acta dermato-venereologica*, **54**, 183.

Precursor keratin protein from human epidermis

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SUMMARY

Prekeratin has been isolated from human epidermis using the method of Matoltsy for bovine nose epidermis. Analysis by sedimentation equilibrium indicates that human prekeratin is heterogeneous in solution. The molecular weight range is from about 200×10^3 to at least 2×10^6 . Polyacrylamide gel electrophoresis shows three polypeptide chains of molecular weights 65,000, 60,000 and 58,000. A lower than average ratio of polar/apolar amino acid residues suggests that hydrophobic interactions are important for stability.

The isolation of prekeratin from bovine snout tissue by Matoltsy (1965) has led to a description of its physical characteristics. Hitherto such studies have been mainly on bovine epidermis and characterization of prekeratin extracted from human tissue by this method is incomplete. Here we describe our present work relating to human epidermal prekeratin.

Matoltsy (1965) in his early work found that the prekeratin extracted by citric acid-sodium citrate buffer, pH 2.6, from bovine snout epidermis appeared monodisperse during ultra-centrifugation and Tiselius electrophoresis and retained the α -helical nature and fibre-forming capacity of the native protein. Prekeratin extractions with citrate buffer thus seemed preferable to the alternative urea extraction which was reported to result in heterogeneous products perhaps very different in nature from the native protein (Rudall, 1952).

More recently O'Donnell (1971) examined solubilized keratin precursor from cow's lip epidermis using formic acid as an extractant. He concluded that the main fraction of fibrous protein extracted by formic acid was the same protein as the epidermin obtained by Rudall (1952) using concentrated urea and the prekeratin extracted by Matoltsy using citric acid-sodium citrate buffer. O'Donnell's

The terms 'prekeratin' and 'precursor keratin protein' are used in the following sense:

'Prekeratin'—initially the term given to fibrous protein extracted from living epidermal cells by citric acid-sodium citrate buffer, pH 2.6 (Matoltsy). Now used more generally for fibrous protein extracted from the living layers of epidermis by other reagents as well. Extraction of the fibrous proteins from stratum corneum requires more drastic measures than those adequate to remove prekeratin.

The major proteins which pack the dead cells of the stratum corneum, the 'soft keratins' are remarkably similar to prekeratin (amino acid composition, subunit mol. wt. by polyacrylamide gel electrophoresis). As there is no synthesis of protein in the stratum corneum there seems little doubt that fibrous proteins in the living cells are 'precursor keratin protein' contributing to the final stabilized keratins. This does not exclude other possible contributions, e.g. from ketatohyalin.

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study also indicated no major homogeneous keratin precursor in cow's lip epidermis but rather a family of closely related proteins, resembling α -keratin of wool.

Further evidence that the urea-extractable proteins and prekeratin of bovine snout epidermis are related is given by Carruthers (1973). Lack of homogeneity has been confirmed by Wissler & Carruthers (1975) in an analysis of prekeratin from bovine snout epidermis by a sedimentation equilibrium method. Our findings indicate that prekeratin extracted from human tissue is similar in nature to that of bovine epidermis and is also not homogeneous in solution. The human prekeratin also has characteristics similar to the major protein fraction extracted from human epidermis by urea (Baynes & Levine, 1976).

MATERIALS AND METHODS

Human thigh skin was removed after leg amputations or at autopsy using a Davol dermatome with disposable head set at a thickness of 0.38 mm. Epidermis was separated from dermis after clamping the skin between metal or glass plates and immersing in a water bath at 60°C for up to 2 min. An alternative method was to scrape the epidermis from skin frozen over dry ice. The epidermis was either used immediately or stored in liquid nitrogen.

Prekeratin was prepared by the method of Matoltsy (1965). Finely minced epidermis was added to 0.1 M citric acid-sodium citrate buffer, pH 2.6 and stirred mechanically for 1 h. The supernatant was decanted and the residue washed with fresh buffer. The combined extract, approx. 15 ml g⁻¹ wet weight of epidermis, was centrifuged twice at 26,000 *g* resulting in a clear, slightly viscous solution. Prekeratin was purified by four serial precipitations at pH 7, 6, 5 and 4.5. The final preparation was dialysed against citric acid-sodium citrate buffer. Protein concentration was estimated by the method of Lowry *et al.* (1951) which was modified to take into account the pH effect of the buffer.

Protein Fraction A was the major protein fraction isolated from human epidermis by urea extraction and gel filtration on a Sephadex G-200 column. Details of the preparation have previously been described (Baynes & Levine, 1976).

Ultracentrifugation

Equilibrium ultracentrifugation runs were carried out in a Beckman Model E Analytical Ultracentrifuge on samples of prekeratin in 0.1 M citric acid-sodium citrate buffer, pH 2.6. Three concentrations (0.25, 0.5 and 1.0 mg/ml) were run at four speeds (12591, 10184, 8225.6 and 6166.4 rev/min) at 10°C using a multichannel equilibrium centrepiece.

Photographs of the Rayleigh interference pattern were taken at various time intervals on Ilford G30 plates. When the fringe pattern remained constant for a period of 3–5 h, photographs were taken for analysis and the speed reduced.

A Fortran computer programme was used to determine the weight average molecular weights, $\bar{M}_w$, from the slope of $\log_{10} J$ vs r^2 using the relation

$$M = \frac{2 R T}{(1 - \bar{v} \rho) \omega^2} 2.303 \frac{(d \log J)}{d r^2}$$

Where *R* is the molar gas constant, *T* is the absolute temperature, $\bar{v}$ is the partial specific volume, ρ is solvent density, ω is angular velocity (radians s⁻¹), *J* is the absolute fringe number and *r* is the radius from the axis of rotation. The partial specific volume was assumed to be 0.730 cm³ g⁻¹ (Matoltsy, 1965).

Polyacrylamide gel electrophoresis

The methods were essentially those of Weber & Osborn (1969) using 10% gels with standard (3%) or occasionally half-standard cross-linkage. Prekeratin solution was dialysed against 0.01 M sodium phosphate buffer (pH 7.0) containing 1% dodecyl sulphate and 1% mercaptoethanol, then heated at 37°C for 3–4 h or at 90°C for 2 min.

Such treatment was found adequate for saturation of SDS binding as judged by Ferguson plots of log mobility against gel concentration (Ferguson, 1964) for standard proteins and prekeratin samples run in a discontinuous system on gels varying from 3 to 12% total acrylamide concentration with 3% cross-linking.

Electrophoresis was carried out for 4 and 2.5 h for the standard and half-standard cross-linked gels respectively at a current of 8 mA per tube. The gels were stained in 1% (w/v) amido black in 7% (v/v) acetic acid for 30 min after cutting them through the bromophenol blue marker band and destained by elution with 7% acetic acid. Gels were scanned on a Beckman Acta CIII recording spectrophotometer, using a Mark 2 Gel Scanner Attachment. Relative mobilities were calculated as the ratio of the distance travelled by the protein to the length of the gel.

Amino acid analysis

Prekeratin was hydrolyzed overnight in 6 M (redistilled) HCl in sealed tubes under nitrogen at 100°C. Analyses were performed on a Jeol JLC 6AH amino acid analyzer.

Tryptophan estimation

Estimation of tryptophan was based on its oxidation by N-bromosuccinimide which results in a decrease in absorption at 280 nm. The procedure was adapted from Blackburn (1968).

The ultraviolet absorption spectrum of 2.5 ml protein solution (1–2 mg/ml) in 8 M urea, 0.05 M sodium acetate buffer, pH 4.0, was obtained in a Beckman spectrophotometer Acta CIII. Oxidation of the tryptophan residues was carried out by successive additions of 5 ml aliquots of 0.02 M N-bromosuccinimide in the same solvent. Similar quantities were added to a blank of the urea buffer, the solutions were well-mixed and the spectrums measured by an overlay scan.

N-Bromosuccinimide was added until the decrease in absorption at 280 nm had reached a maximum, i.e. complete oxidation. The initial A_{280} of the unoxidized protein was corrected for dilution to the final concentration of protein. Tryptophan content was estimated by the formula:

$$\text{Tryptophan (mg)/mg protein} = \frac{f \Delta A_{280}}{c l E_{280}}$$

where c is the final concentration of protein (mg/ml); l the path length of the cell (1 cm); ΔA_{280} (corrected A_{280} unoxidized protein) – (A_{280} oxidized protein); E_{280} , extinction coefficient of tryptophan at a concentration of 1 mg/ml (29.6); and f is an empirical factor arising from the fact that oxidation does not reduce the A_{280} to zero. A value of 1.31 was used.

Electron microscopy

Prekeratin solution was dialysed against distilled water and a thin film allowed to dry on a carbon-coated collodion grid. The specimen was stained in 1% phosphotungstic acid, pH 3.5 followed by 1% uranyl acetate, pH 4.2 for 15 min in each. The preparation was examined in an AEI EM6G.

RESULTS AND DISCUSSION

The yield of prekeratin varied from 1 to 3% of the wet weight of epidermis from different individuals.

Results from the same individual were reproducible to 2 figures. Both whole skin and epidermis have been stored for up to 6 weeks in liquid nitrogen without apparent deterioration. This is in contrast to attempted extraction following storage of skin at -20°C where the product was an insoluble gel intractable to isolation of the prekeratin.

Sedimentation equilibrium

Equilibrium analytical ultracentrifugation yielded plots of $\log J$ against r^2 which were markedly curved, the slope increasing towards the position of the cell bottom. The weight average molecular weights, $\bar{M}_w$ calculated from the plots are shown in Figs 1 and 2. Lack of homogeneity is demonstrated by the dependence of the weight average molecular weight range on the velocity of the run.

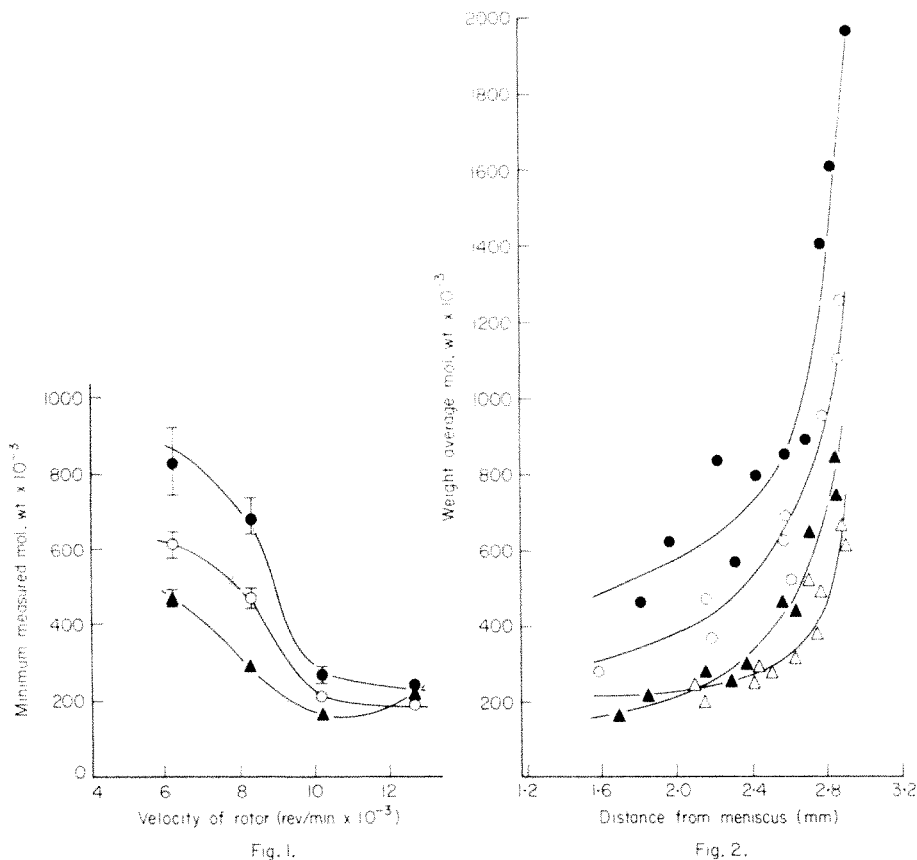


FIGURE 1. Minimum measured molecular weight change with speed. (●) 0.25 mg/ml; (○) 0.50 mg/ml; (▲) 1.0 mg/ml.

FIGURE 2. Variation of molecular weight with distance from the meniscus. Speed of rotor (rev/min $\times 10^{-3}$): (●) 6166; (○) 8225; (▲) 10,183; (Δ) 12,591.

In Fig. 1, the data presented show the relation between the minimum measured molecular weight and the velocity of the run for three concentrations of protein. Increasing the speed of the rotor has the effect of sedimenting a greater fraction of large molecular weight material thus accounting for the decrease in measured minimum molecular weight. Convergence of the speed-dependent curves is suggested leading to a value in the region of 200×10^3 for the minimum molecular weight.

In Fig. 2 the complete data are plotted differently. Here, the average molecular weight is given as a function of the distance from the meniscus for each of four speeds. The average molecular weight is seen to vary with speed since sedimenting of material occurs to a greater degree as the speed is increased. Therefore, at any given distance from the meniscus, the lower the speed, the higher the average molecular weight. The upper limit of molecular weight is uncertain but would appear to be above 2×10^6 .

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis in dodecyl sulphate carried out on a prekeratin sample within 24 h of its preparation showed three monomer species present corresponding to polypeptides of molecular weights 65,000, 60,000 and 52,000 (Fig. 3). A sample of crude extract of prekeratin before any precipitation steps gave virtually the same pattern. These were the only constituents of freshly-prepared prekeratin. On the basis of relative stain intensity, most of the material appears to be in bands (1) and (2). Band (3) was fainter and less sharp. Both standard and occasional use of half-standard cross-linkage gave the same result. Repeated runs indicated a high degree of reproducibility for bands (1) and (2). (Standard deviations between 1.5 and 2.5%.) Band (3) was more variable and may possibly be a break-down product.

Table 1 gives the molecular weights obtained by polyacrylamide gel electrophoresis for various epidermal proteins. There is a general overall similarity with particularly close values for the human prekeratin and the stratum corneum protein of rat.

When prekeratin in citrate buffer was stored for several weeks at 4°C before electrophoresis, new bands appeared of lower molecular weights. The breakdown may be due to proteolysis and this aspect was not investigated further. Only freshly-isolated prekeratin or freeze-dried material, which was unchanged by the treatment, was used for analysis.

In previous work with urea-extracted protein Fraction A (Baynes & Levine, 1976) polyacrylamide

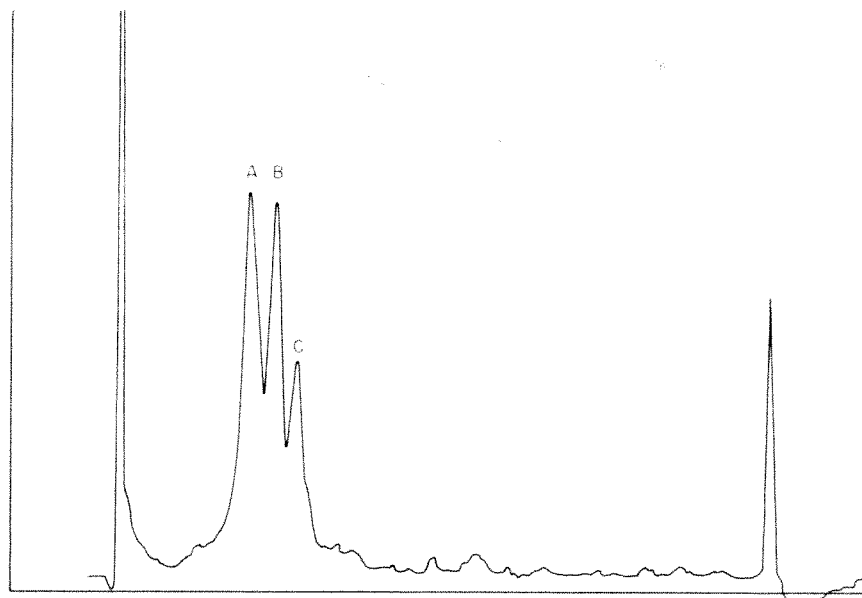


FIGURE 3. Polyacrylamide gel electrophoresis of human prekeratin. Scan of stained gels at 580 nm. Peak A, mol. wt 65,000; peak B, mol. wt 60,000; peak C, mol. wt 58,000.

TABLE 1. Molecular weights of the major polypeptides by polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis in dodecyl sulphate was carried out on freshly prepared prekeratin as described in the text. Also shown for comparison are results for urea-extracted protein fraction A, bovine prekeratin and rat stratum corneum protein

Epidermal protein	Mol. wt of main bands		Relative proportion
	(1)	(2)	
Human prekeratin	65,000	60,000	
Urea-extracted fraction A	(3) 52,000		1:1*
(Baynes & Levine, 1976)	77,000	63,000	1:2
Bovine prekeratin	72,000	60,000	1:2
(Skerrow, 1974)			
Bovine prekeratin	58,000	49,000	1:2
(Steinert, 1975)			
Rat stratum corneum	68,100	61,000	—
(Shimizu <i>et al.</i> , 1974)			
Rat stratum corneum	67,000	60,000	1:1
(Huang <i>et al.</i> , 1975)			

* Refers to main bands (1) and (2). Band (3) was fainter and more variable, possibly a breakdown product.

gel electrophoresis revealed two main bands molecular weights 77,000 and 63,000, but numerous additional subunits present indicated a range of molecular weights from 14,000 to 160,000.

Absorption spectra and amino acid composition

Both prekeratin and urea-extracted protein Fractions A gave anomalous ultraviolet absorption spectra with ratios of A_{280}/A_{260} equal to or less than unity, 1.00 and 0.72 for prekeratin and Protein Fraction A, respectively. The absorption spectrum of prekeratin is shown in Fig. 4. From the amino acid compositions (Table 2) the ratios would be expected to be less than the average protein. Calculation

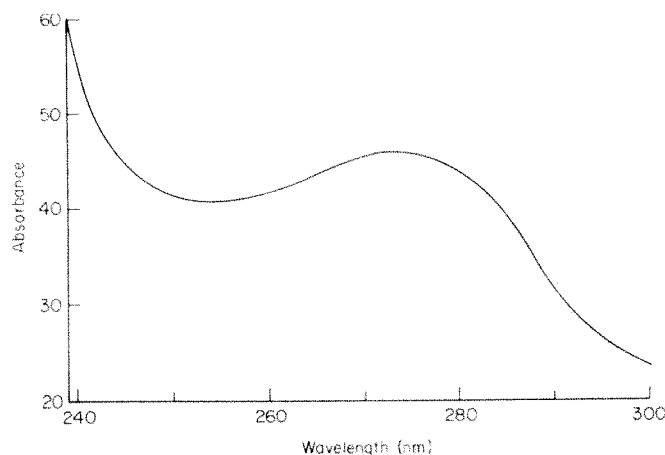


FIGURE 4. Absorption spectrum of human prekeratin.

TABLE 2. Amino acid analysis of epidermal proteins. Human prekeratin was subjected to amino acid analysis as described in the text. Results are expressed as residues/100 residues and represent the average of duplicate analyses. Results for other epidermal proteins are given for comparison

Amino acid	Human prekeratin	Human urea extract protein A (Baynes & Levine, 1976)	Human stratum Corneum protein (Baden <i>et al.</i> , 1975)	Bovine pre-keratin (Baden <i>et al.</i> , 1971)	New born rat stratum corneum protein (Shimizu <i>et al.</i> , 1974)
Aspartic	8.8	9.5	9.4	9.1	8.6
Threonine	3.7	4.9	3.9	4.0	3.8
Serine	13.0	8.6	11.5	11.1	12.8
Glutamic	13.4	13.6	13.7	14.1	13.9
Proline	1.7	4.9	2.3	1.4	1.6
Glycine	20.6	12.3	18.4	16.4	21.5
Alanine	5.7	7.7	5.5	6.7	4.2
Half-cystine	0.4	1.2	—	0.6	0.7
Valine	3.5	4.7	3.7	4.0	4.2
Methionine	1.2	2.4	1.5	1.3	1.0
Isoleucine	3.2	2.7	3.2	3.5	3.6
Leucine	7.6	8.6	8.4	9.2	7.7
Tyrosine	3.6	2.4	3.2	2.8	3.7
Phenylalanine	3.3	3.5	3.4	3.6	3.3
Lysine	4.1	6.1	4.3	5.1	5.0
Histidine	1.1	2.1	1.0	1.0	0.2
Arginine	5.0	4.9	4.7	6.1	4.3
Tryptophan	0.7	1.2	—	—	—

of the contributions to absorbance at 280 and 260 nm by the aromatic amino acids gives a value of 1.3. It has been shown that Protein Fraction A is not associated with nucleic acids or nucleotides (Baynes & Levine, 1976). The nature of a possible non-protein moiety to account for the anomalous absorption is yet unknown.

The amino acid composition of human prekeratin can be compared with results reported by other authors for various epidermal proteins. Human and bovine prekeratins are very similar as are also the prekeratins and stratum corneum proteins. Steinert (1975) found that urea-extracted polypeptides from viable and non-viable layers of bovine epidermis were indistinguishable.

The proportion of hydrophobic residues in the prekeratin molecule is higher than average. The ratio of polar to apolar residues in prekeratin is 1.15 compared with the average value of 1.5 for proteins generally (Hatch, 1965). A low ratio is often an indication that the protein consists of subunits associated by noncovalent forces such as hydrophobic bonding. It is likely that the polypeptide chains in the native state are held together by non-covalent forces and when the cell is ruptured by treatment with citrate buffer or urea, the molecule tends to dissociate.

Prekeratin is rich in asp, ser, glu, gly and leu. The first four are non-essential amino acids, the so-called 'cheap' amino acids which are readily metabolized. The exception is leucine, one of the 'indispensable' amino acids required in the diet. One might speculate as to a specific role for leucine in the architecture of a protein that functions mainly as a protective barrier. It has been shown in studies of synthetic block copolypeptides of glu and leu that leucine is very stable in the α -helical structure (Jones, Patrick & Phillips, 1976). Such block copolypeptides form very stable aggregates in aqueous solution. The aggregates are not dissociated under conditions which would denature globular proteins.

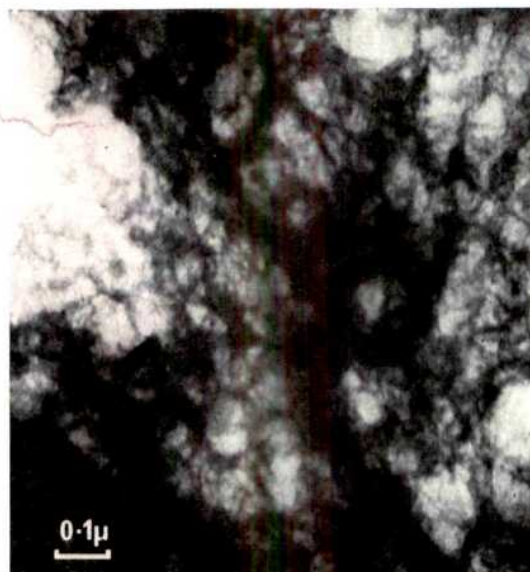


FIGURE 5. Electron micrograph of reconstituted human prekeratin.

Electron microscopy

Fig. 5. shows the fibrous nature of aggregates of human prekeratin reconstituted from solution. It resembles prekeratin of bovine epidermis prepared in a similar way by Matoltsy (1965).

CONCLUSIONS

Although models of the basic subunit of epidermal prekeratin have been suggested, there is as yet no conclusive evidence for the structure of the molecule. Skerrow *et al.* (1973), from results of studies of polypeptide fractions obtained from bovine epidermal prekeratin by enzymic digestion, reported evidence for a probable triple chain of polypeptides. However, the possibility of a double chain could not be excluded. The molecular weight of a triple chain would be of the order of 200×10^3 .

Our results by polyacrylamide gel electrophoresis suggest that there are two or three polypeptide chains making up the subunit. From our sedimentation equilibrium experiments it has been shown unequivocally that human epidermal prekeratin is heterogeneous in solution. Previous reports describing bovine prekeratin have been contradictory. Homogeneity was suggested by Matoltsy (1965) whereas experiments of Wissler & Carruthers (1975), by the method of sedimentation equilibrium, showed that bovine prekeratin was not homogenous. A minimum molecular weight in the region of 200×10^3 could represent the basic subunit of human prekeratin.

The nature of the amino acid residues supports the postulate that non-covalent forces, largely hydrophobic, confer stability on the polypeptide structure and on the complex aggregates *in situ*.

The arrangement that has been postulated for the basic structural elements in hard mammalian keratin consists of eleven double helices, two at the centre and nine at the periphery (Fraser *et al.*, 1972). The filaments of soft α -keratin and the microfibrils of hard α -keratin are comparable in diameter. If the hard and soft α -keratins are structurally similar, the molecular weight for the prekeratin unit would be in the region of 2×10^6 .

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REFERENCES

- BADEN, H.P., GIFFORD, A.M. & GOLDSMITH, L.A. (1971) The precursor of the α -fibrous protein of epidermis. *Journal of Investigative Dermatology*, **56**, 446.
- BADEN, H.P., GOLDSMITH, L.A. & LEE, L.D. (1975) The fibrous proteins in various types of ichthyosis. *Journal of Investigative Dermatology*, **65**, 228.
- BAYNES, J. & LEVINE, M. (1976) Urea-extractable protein from human epidermis. *Biochimica et Biophysica Acta* **439**, 107.
- BLACKBURN, S. (1968) *Amino Acid Determination—Methods and Techniques*, pp. 190–192. E. Arnold Ltd, London.
- CARRUTHERS, C. (1973) On the relationship between the urea-soluble and citric acid-soluble proteins of bovine snout epidermis. *British Journal of Dermatology*, **89**, 477.
- FERGUSON, K.A. (1964) Starch-gel electrophoresis—application to the classification of pituitary proteins and polypeptides. *Metabolism: Clinical and Experimental*, **13**, 985.
- FRASER, R.D.B., MACRAE, T.P. & ROGERS, G.E. (1972) *Keratins*, Chp. 5. Charles C. Thomas, Springfield, Illinois.
- HATCH, F.T. (1965) Correlation of amino acid composition with certain characteristics of proteins. *Nature*, **206**, 777.
- HUANG, L.Y., STERN, I.B., CLAGETT, J.A. & CHI, E.Y. (1975) Two polypeptide constituents of the major protein of the cornified layer of newborn rat epidermis. *Biochemistry*, **14**, 3573.
- JONES, M.N., PATRICK, C.P. & PHILLIPS, M.C. (1976) The aggregation of block copolypeptides in aqueous solution. *Journal of Colloid and Interface Science*, **55**, 116.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265.
- MATOLTSY, A.G. (1965) Soluble prekeratin. In: *Biology of the Skin and Hair Growth* (Ed. by A.G. Lyne and B.F. Short), pp. 291–305.
- O'DONNELL, I.J. (1971) A search for a simple keratin—the precursor keratin proteins from cow's lip epidermis. *Australian Journal of Biological Sciences*, **24**, 1219.
- RUDALL, K.M. (1952) The proteins of the mammalian epidermis. *Advances in Protein Chemistry*, **7**, 253.
- SHIMIZU, T., FUKUYAMA, K. & EPSTEIN, W.L. (1974) Partial purification of proteins isolated from mammalian cornified cells. *Biochimica et Biophysica Acta*, **359**, 389.
- SKERROW, D. (1974) The Structure of prekeratin. *Biochemical and Biophysical Research Communications*, **59**, 1311.
- SKERROW, D., MATOLTSY, G. & MATOLTSY, M.N. (1973) Isolation and characterisation of the α -helical regions of epidermal prekeratin. *Journal of Biological Chemistry*, **248**, 4820.
- STEINERT, P.M. (1975) The extraction and characterisation of bovine epidermal α -keratin. *Biochemical Journal*, **149**, 39.
- WEBER, K. & OSBORN, M. (1969) The reliability of molecular weight determination by dodecyl sulphate–polyacrylamide gel electrophoresis. *Journal of Biological Chemistry*, **244**, 4406.
- WISSLER, F. & CARRUTHERS, C. (1975) Heterogeneity of bovine snout epidermal prekeratin. *British Journal of Dermatology*, **92**, 647.

Weber-Christian syndrome in infancy

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SUMMARY

A review of Weber-Christian syndrome in infancy is presented along with the report of two cases. Both infants had low serum properdin levels, and one patient showed remissions of his disease when he was T-lymphocytopenic. This suggests that the T-lymphocyte may have an important role in the pathogenesis of Weber-Christian syndrome.

Relapsing, febrile, nodular, non-suppurative panniculitis or Weber-Christian syndrome was first described by Pfeifer (1892). Since that time less than 60 cases of this unusual panniculitis have been reported in children under 12 years of age. The first case report was by Gilchrist & Ketron (1916), who described an 8-year-old female who had non-painful, non-suppurative, nodular skin lesions on her thighs, legs, and ankles which healed with secondary atrophy and were associated with fever and 'swelling of the joints'. The child was treated with potassium iodide without effect, and went on to have several other attacks of fever and nodular panniculitis.

Weber (1925) described an adult with 'relapsing nodular non-suppurative panniculitis', Christian (1928) added the adjective 'febrile' to the name of this syndrome, and Brill (1936) gave the disorder its present name. Cummins & Lever (1938) described two children with Weber-Christian syndrome, and proposed that the panniculitis had three stages pathologically.

Carol, Prakken & Zwijndregt (1941) reported the first case of Weber-Christian syndrome in infancy. They observed a 6-month-old female with generalized subcutaneous nodules associated with fever, palpable spleen, and early signs of rickets. Six months later the child developed hydrocephalus, and continued to have bouts of Weber-Christian syndrome over the next 5 years.

Sanford, Eubank & Stenn (1952) took care of an 8½ month old male with generalized subcutaneous nodules, hepatomegaly and fever. The infant received multiple antibiotic therapy, blood transfusions, and ACTH without benefit, and suddenly died on the 10th day of hospitalization. Permission for autopsy was refused.

Vestermark (1966) described a newborn girl with skin nodules on her buttocks and left arm, accompanied by hepatomegaly. At 2 weeks of age the infant developed pneumonia and thrush. Six months later persistent hepatomegaly with elevated liver function enzymes, leukocytosis, and an elevated erythrocyte sedimentation rate were present.

Kunzer *et al.* (1970) reported a 7-month-old male infant with generalized skin nodules associated with vomiting, ecchymoses, prolonged clotting studies, and decreased platelets. He recovered after

antibiotic therapy, prednisone, and transfusions of blood and fresh frozen plasma. One month later he was readmitted to the hospital where he died of septicæmia and disseminated intravascular coagulation despite heparin, antibiotics, prednisone, blood transfusions, and oxyphenbutazone. At autopsy widespread fibrin thrombi with hæmorrhage were noted as well as a large thymus, normal spleen, periadrenal fat inflammation with foamy macrophages, and increased Kupffer cells in the enlarged liver.

Masicowa & Wojcienchowska (1971) cared for an 18-month-old girl with recurrent subcutaneous nodules since 2 months of age associated with lymphadenopathy, hepatosplenomegaly, muscle pains and contractures, and failure to thrive. Laboratory investigations included: negative lupus erythematosus cell preparation, cryoglobulins—1:64, and an elevated erythrocyte sedimentation rate during attacks of her disease. On prednisone therapy the child's symptoms abated. A year and a half later she was having fewer and less severe attacks of Weber-Christian syndrome.

CASE REPORTS

Case 1

This 6-month-old Caucasian male infant was the 2.7 kg product of a full term normal pregnancy, labour and delivery. He was in good health until about 9 weeks of age when he developed a fever within 24 h after diphtheria-pertussis-tetanus injection (DPT) and oral polio vaccine. His fever responded to acetaminophen but recurred the next day, and an erythematous, indurated lesion was noted on his left buttock at the site of his injection. Over the next few days other indurated, erythematous nodules appeared on his left forearm, left foot, and right buttock. He was hospitalized, and was treated with methicillin, gentamicin, and clindamycin without improvement. Two of the skin lesions were incised and drained, and one of them grew an anaerobic streptococcus. A skin biopsy was inter-

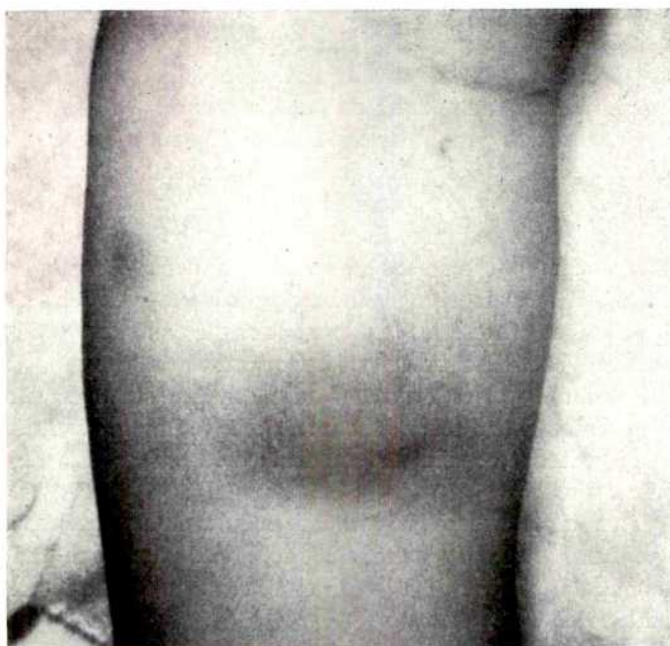


FIGURE 1. Large, erythematous, tender subcutaneous nodule on the forearm.

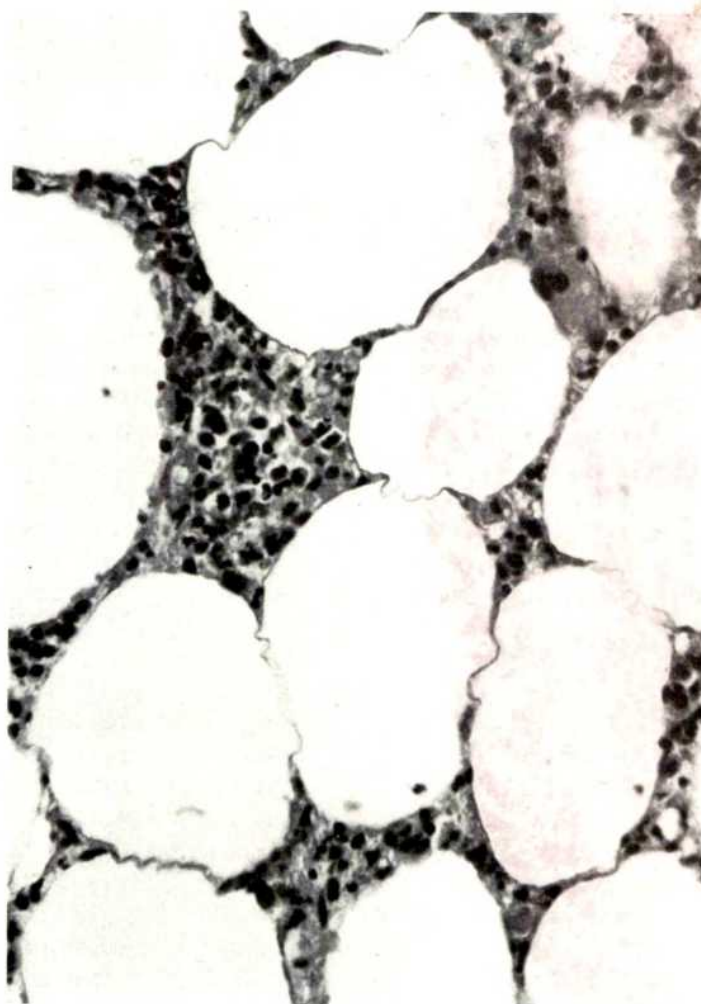


FIGURE 2. Case 1. Biopsy of a subcutaneous nodule over 2 days old showing focal areas of fat cell necrosis with a polymorphonuclear leukocyte infiltrate scattered throughout the fat lobule (H & E, $\times 500$).

preted as 'neutrophilic panniculitis', and systemic corticosteroids were begun with apparent improvement of the child's clinical condition. At about 5½ months of age, however, he developed new lesions on his arms and insteps. He was again admitted to the hospital, where a diagnosis of bilateral otitis media with pharyngitis was made. He was placed on penicillin, gentamicin, and systemic corticosteroids with resolution of his upper respiratory tract symptoms but persistence of his skin lesions and intermittent fever. Since he was not responding to therapy, the infant was transferred to University Hospitals.

On admission his physical examination was essentially normal except for 8–10 indurated, erythematous, slightly warm, tender nodules distributed over his buttocks, arms and legs (Figs 1 & 2).

Initial laboratory studies included a haemoglobin of 9.9 g%, white blood count—26,500/mm³ with 62% polymorphonuclear leukocytes, 21% lymphocytes, 15% monocytes, and 1% eosinophils,

platelets $411,000/\text{mm}^3$, and erythrocyte sedimentation rate 44 mm/h . The following laboratory studies were normal: chest X-ray, skull X-rays, flat X-ray of the abdomen, electrocardiogram, lupus erythematosus cell preparation, antinuclear factor, latex fixation test, VDRL, cryoglobulins, nitroblue tetrazolium test, stool analysis, urinalysis, and cultures of the skin, blood, stool, urine and throat. The C3 was $245 \text{ mg}\%$ (normal range $90\text{--}180 \text{ mg}\%$), C4 was $30.4 \text{ mg}\%$ (normal range $20\text{--}50 \text{ mg}\%$), and the immunoglobulins IgG & IgA were normal with an elevated IgM. The properdin level performed in the manner described by Pillemar *et al.* (1954) was less than 1 unit/ml (normal control 8 units/ml).

Over the next several days new skin lesions appeared associated with spiking fevers to 40.6°C rectally. The infant was placed on prednisone (2 mg/kg/day) with some improvement. On the 6th day he developed tachypnoea and dyspnoea associated with fever. A chest X-ray showed an infiltrate in the right upper and middle lobes as well as in the left lower lobe, and he was started on intravenous ampicillin. One blood culture grew an alpha-streptococcus, although repeat blood cultures were sterile. He was changed to oral penicillin with gradual clearing of his chest X-ray. On the 9th hospital day his skin biopsy site drained 5 ml of sterile white exudate, and 2 days later a nodule on his right foot suppurated yielding 3 ml of sterile pus. Otherwise, the child continued to improve; his prednisone was tapered, and he was sent home.

Since discharge he has had other attacks of fever and malaise associated with tender, erythematous skin lesions which usually respond to a short course of prednisone. Further laboratory tests including a serum alpha-1-antitrypsin level, amylase, lipase, liver function enzymes, and a follow-up chest X-ray were normal.

Case 2

This 3-month-old Caucasian male infant was the 3.8 kg product of a full term normal pregnancy, labour, and delivery. At about 3 weeks of age he had a fever associated with an erythematous, indurated, tender subcutaneous nodule on his left thigh which was treated with intramuscular penicillin. At 5 weeks of age he was hospitalized because of a recurrence of fever associated with similar skin lesions. A biopsy at that time was interpreted as acute panniculitis (Rothman-Makai type), and he was treated with penicillin, erythromycin, sulphafurazole, and prednisone ($2\text{--}4 \text{ mg/kg/day}$). Since he was not responding to therapy, he was transferred to University Hospitals.

The admission physical examination was unremarkable except for a palpable liver and spleen 1–2 cm below the costal margin. On the third hospital day multiple, erythematous, indurated, 0.5–1.5 cm nodules appeared on his arms, legs, buttocks, trunk and neck associated with a fever of $38\text{--}39^\circ\text{C}$ rectally. The skin lesions were transient lasting only a few days, and left no residual scars.

Initial laboratory studies included: haemoglobin $7.9 \text{ g}\%$, reticulocyte count 1.3% , white blood count $15,200/\text{mm}^3$ with 63% polymorphonuclear leukocytes, 17% lymphocytes, 2% atypical lymphocytes, 8% monocytes, and 10% bands, platelets $471,000/\text{mm}^3$, serum iron $15 \mu\text{g}\%$, and erythrocyte sedimentation rate 36 mm/h . An excisional skin biopsy was interpreted as acute panniculitis consistent with early Weber-Christian syndrome (Fig. 3). Other laboratory studies were normal: Electrolytes, glucose, blood urea nitrogen, calcium, phosphorus, creatinine, serum and urine amylase, triglycerides, cholesterol, liver function enzymes, urinalysis, antinuclear factor, lupus erythematosus cell preparation, latex fixation test, ASO titres, cytomegalic inclusion virus, herpes virus, cryoglobulins, cold agglutinins, direct Coombs' test, heterophile, hepatitis associated antigen, VDRL, immunoglobulins (including IgE), serum protein electrophoresis, metabolic screen of the urine, chest X-ray, intravenous pyelogram, barium enema, bone age determination, flat X-ray of the abdomen, electrocardiogram, and cultures of the urine, blood, cerebrospinal fluid, stool, skin, and throat. The C3 was $233 \text{ mg}\%$ (normal range $90\text{--}180 \text{ mg}\%$) and the C4 was $22 \text{ mg}\%$ (normal range $20\text{--}50 \text{ mg}\%$). Direct immunofluorescence of the skin for IgG, IgM, complement, and fibrinogen was

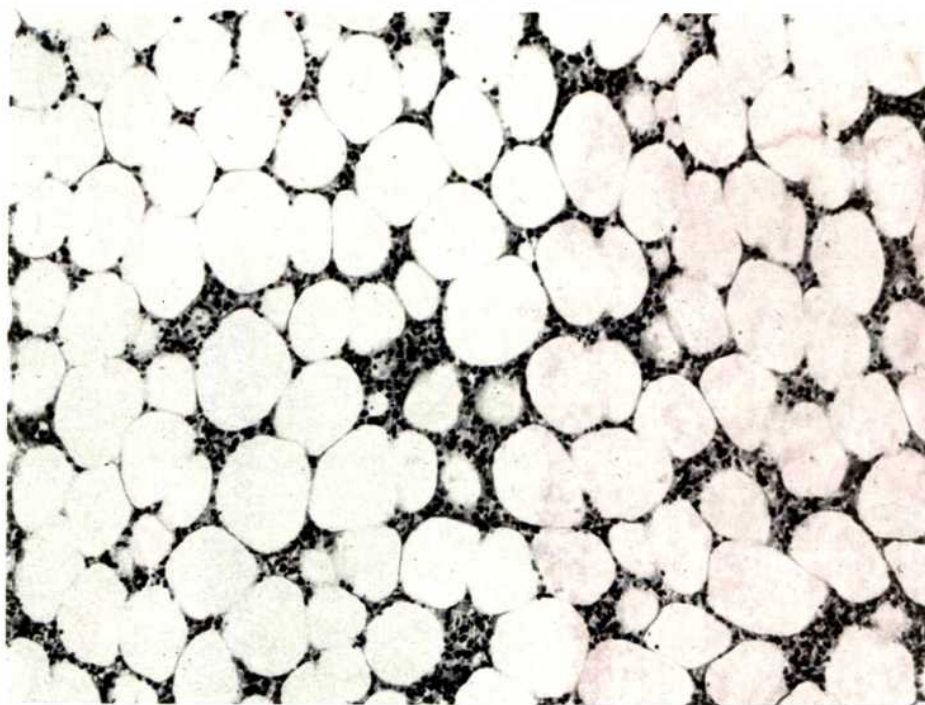


FIGURE 3. Case 2. Acute panniculitis with marked polymorphonuclear leukocyte infiltration of the fat lobule from a biopsy of a subcutaneous nodule over 2 days old (H & E, $\times 250$).

negative, and intradermal skin tests with 5 units PPD, diphtheria toxin, candida, and streptokinase with streptodornase were all negative. The properdin level performed in the manner described by Pillemar *et al.* (1954) was less than 1 unit/ml (normal control 4 units/ml).

The patient's hospital course was extremely stormy. On the 14th hospital day he developed intermittent haem-positive stools associated with abdominal distention and fever. A barium enema at that time was normal, and upper gastrointestinal X-rays showed distended loops of small bowel. The child was begun on ampicillin and gentamicin. On this therapy his fever seemed to resolve, but he continued to have haem-positive stools and abdominal distention. A laparotomy was performed, and a necrotic segment of jejunum and ileum was excised. The postoperative course was complicated by sepsis and suspected disseminated intravascular coagulation, which was treated with five exchange transfusions, ampicillin, gentamicin, and methylprednisolone. On the fourth postoperative day he developed abdominal distention requiring repair of his intestinal anastomotic site along with externalization of the bowel. Four days after closure of the abdominal wall a morbilliform eruption appeared over his trunk and extremities associated with a swollen right knee joint and elevated liver function enzymes. Gentamicin and ampicillin were discontinued, and chloramphenicol was begun. Over the next 48 h he became febrile with a new crop of subcutaneous nodules, although his morbilliform rash slowly resolved over the next few days. Chromosome analysis performed 5 days later to rule out a graft versus host reaction was normal. On the 48th and 55th hospital days he became febrile, and a new crop of subcutaneous nodules appeared over his trunk and extremities. He continued to feed poorly, and hyperalimentation was begun. Several weeks later he developed septicaemia, and was treated with ampicillin and gentamicin. Slowly the child began to gain weight, and was discharged from the hospital.



FIGURE 4. Case 2. Large, erythematous, subcutaneous nodule which persisted for over 1 week leaving residual hyperpigmentation.

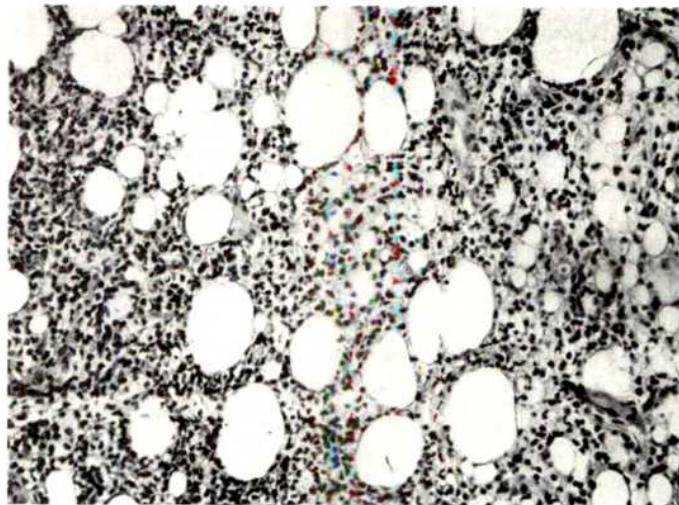


FIGURE 5. Case 2. Predominantly mononuclear infiltration of the panniculus with occasional foamy-appearing histiocytes (H & E, $\times 55$).

Persistent hepatosplenomegaly, elevated liver function enzymes, and leukocytosis were present on follow-up examination. He continued to have attacks of fever associated with skin lesions every 2-3 weeks. These subcutaneous nodules were larger than before, and persisted for over a week leaving slightly hyperpigmented areas (Fig. 4). He was re-hospitalized when a liver biopsy showed severe fatty changes and chronic passive congestion without any evidence of foamy histiocytes. These changes were interpreted as consistent with chronic malnutrition, thought to be secondary to previous surgery. A skin biopsy of a 3-day-old lesion was interpreted as Stage 2 Weber-Christian syndrome (Fig. 5). Direct immunofluorescence of the skin for IgG, IgA, IgM, complement, and fibrinogen was negative. Further laboratory studies including serum toxoplasmosis, alpha-1-antitrypsin, glucose-6-phosphate dehydrogenase, lipase, and a Rebuck skin window were all normal. The properdin level was less than 1 unit/ml (normal control 4 units/ml), and a quantitative analysis of the stool demonstrated fat malabsorption. Repeat intradermal skin tests with 5 units PPD, mumps, trichophyton, streptokinase-streptodornase, and candida were non-reactive, while *in vitro* lymphocyte stimulation tests to phytohaemagglutinin, concanavalin A, and pokeweed antigen were all diminished. T and B cell determinations showed 21% T-lymphocytes by E-rosettes (normal control 40.5%) and 14.5% B-lymphocytes by EAC rosettes (normal control 23.5%) during remission of his disease.

About one month later after the child was gaining weight on a high carbohydrate low fat formula (Probana) with frequent feedings, he had another attack of subcutaneous nodules associated with fever up to 40°C. At that time his T-lymphocytes were 53.5% by E-rosettes (normal control 55.8%) and his B-lymphocytes were 15.5% by EAC-rosettes (normal control 18.5%). He now began to have weekly attacks of fever and subcutaneous nodules associated with malaise, irritability, and poor feeding. A trial of dapsone was initiated beginning with 3 mg per day and increasing slowly to 9 mg per day. Despite dapsone therapy the child developed spiking fevers and subcutaneous nodules associated with a painful, swollen, left knee joint. This was tapped, and showed 4900 RBC's/hpf and 114,000 WBC's/hpf with 98% neutrophils and 2% mononuclear cells. No organisms were seen on Gram stain. X-rays of the knees showed an effusion of both joints. The child was given 10 mg/day prednisone and intravenous methicillin. By the next morning he was afebrile with marked improvement in the mobility of his left knee. Cultures from the joint aspirate were sterile, and methicillin and dapsone were stopped after 2 days. The joint fluid amylase and lipase levels were normal, as were the serum levels of amylase and lipase and the 24-h urine collection for amylase.

At present the child is being maintained on prednisone 2 mg/kg four times daily to prevent exacerbations of his Weber-Christian syndrome. He is gaining weight on a low fat diet, but still has hepatosplenomegaly with leukocytosis, elevated liver function enzymes, and high erythrocyte sedimentation rate.

DISCUSSION

Weber-Christian syndrome is a rare disorder in infancy and childhood. The true incidence is not known, since mild cases may not seek medical attention (Beerman, 1953), or may be passed off as recurrent insect bites or bruises.

Symptoms

Most infants reported in the English and European medical literature have had systemic symptoms, and two of them have died (Table 1). This is in contrast to the usually mild clinical course of Weber-Christian syndrome in older children and adults. Malaise and irritability are quite common during acute episodes, and poor appetite and weight loss may be present. Six of seven infants had generalized tender, subcutaneous nodules, six had fever, five had some secondary atrophy at the site of their skin lesions, and one had suppuration. Hepatomegaly with or without splenomegaly was demonstrated in

TABLE 1. Weber-Christian syndrome in infancy

Author	Age (months)	Duration	Sex	Fever	Pain	Distribution	Other	WBC	ESR	Amylase	Lipase	Biopsy	Treatment	Recurrences
Carol <i>et al.</i> (1941)	6	weeks	F	+	+	Generalized	Splenomegaly, hydrocephalus	—	—	—	—	Stage 2	Symptomatic	Yes (followed 5 years)
Sanford <i>et al.</i> (1952)	8.5	2.5 months	M	+	—	Generalized (2° atrophy)	Hepatomegaly, possible cryoglobulins	3300	—	—	—	Stage 2	Antibiotics ACTH	Died (autopsy refused)
Vestermark <i>et al.</i> (1966)	Newborn	None	F	—	+	Buttocks, arm (2° atrophy)	Hepatomegaly, pneumonia, thrush	13,850 30,200	66– 55	—	—	Stages 2 and 3	Penicillin	Persistent hepatomegaly at 6 months
Kunzer <i>et al.</i> (1970)	7	Short time	M	+	+	Generalized (2° atrophy)	Vomiting, hepatomegaly (?), ecchymoses	—	—	—	—	Stage 2	Antibiotics steroids	Died of sepsis
Manicova <i>et al.</i> (1971)	18	16 months	F	+	+	Generalized (2° atrophy)	Hepatosplenomegaly, lymphadenopathy, failure to thrive, cryoglobulins 1:64	5800 9800	15– 137	—	—	Stage 1	Antibiotics steroids	Yes (followed 3 years), attacks less severe
Present report Case 1	6	4 months	M	+	+	Generalized (2° atrophy, suppurative)	Pulmonary infiltrates (?)	26,500 34,100	44	152	51	Stage 1	Antibiotics steroids	Yes (followed over a year)
Present report Case 2	3	2 months	M	+	+	Generalized	Hepatosplenomegaly, bowel perforation, joint effusions, failure to thrive	6800– 57,400	36	50	34	Stages 1 and 2	Antibiotics steroids exchange transfusions dapsone	Yes (followed over a year)*

* Since this report was written this child has died.

five infants. Joint effusions, bowel perforation, and possible pulmonary involvement have not been reported previously in infants. Adults, on the other hand, have been described with hepatosplenomegaly and other systemic symptoms (Albrechtsen, 1960; Milner & Mitchinson, 1965) including joint symptoms (DeLor & Martz, 1955; Goldberg & Ritzmann, 1958), involvement of the bowel (Schoen, Reingold & Meister, 1958), and pulmonary lesions (Schoen *et al.*, 1958; Arnold & Bainborough, 1963).

Laboratory

No laboratory test is diagnostic of this disease. Infants often have a low haemoglobin, and the white blood count has ranged from $3300/\text{mm}^3$ to $57,400/\text{mm}^3$ with or without a shift to the left. The amylase, lipase, cholesterol, and triglycerides have been normal, while the erythrocyte sedimentation rate (ESR) is often elevated. Cryoglobulins have been reported in two infants, but were not present in either of our patients. Bacterial cultures are usually sterile except during periods of superinfection. The properdin level was low, the C3 elevated, and the C4 normal in both cases during acute episodes of Weber-Christian syndrome. This may indicate a defect in the alternate complement pathway, which may help to account for both infants' susceptibility to infection (Mayer, 1973). Further evaluation is needed to determine the significance of this finding.

Pathology

Weber-Christian syndrome has three stages pathologically (Lever & Lever, 1975). The first stage is transient, and involves polymorphonuclear cell infiltration of the fatty tissue with fat cell degeneration without abscess formation. During the second stage there is a histiocytic infiltration of the panniculus with phagocytosis of fatty tissue debris and the formation of characteristic 'lipophages'. The inflammatory process usually spares the epidermis and dermis, and blood vessels are affected secondarily as the inflammation in the fat lobules extends peripherally to involve them. Finally, fibroblastic proliferation and collagen deposition occur in areas of inflammation (stage 3). These changes frequently overlap, so that one commonly finds stages 2 and 3 together.

Recently there has been an attempt to classify the panniculitides into lobular and septal types (Reed, Clark & Mihm, 1973). Weber-Christian syndrome and lipogranulomatosis subcutanea of Rothman-Makai fall into the lobular type, while erythema nodosum and its variants are of the septal variety. These changes, however, are not diagnostic, and clinicopathological correlation is necessary. Furthermore, the pathology of Weber-Christian syndrome and lipogranulomatosis of Rothman-Makai is considered identical by many dermatopathologists (Lever & Lever, 1975). The distinguishing features of these two variants of the same disease process or processes are therefore clinical. Rothman-Makai panniculitis is classically a self-limited, benign disorder characterized by one to several nodular lesions which appear without fever or systemic symptoms and gradually resolve without recurrence. Weber-Christian syndrome, on the other hand, typically presents as crops of subcutaneous nodules associated with fever, systemic symptoms, and recurrences.

Both our cases initially had stage 1 Weber-Christian syndrome histologically, suggesting that the polymorphonuclear infiltration of the panniculus is not always a transient phenomenon. In Case 2 the character of the lesions changed appreciably with time, and a biopsy of a three-day-old lesion when the child was 14 months of age showed histiocytic and lymphocytic infiltration of the fat lobule with occasional foamy histiocytes.

Differential diagnosis

Fever associated with subcutaneous nodules in infancy may be seen with insect bites, abscesses or cellulitis, thrombophlebitis, traumatic or factitial fat necrosis, disseminated lipogranulomatosis (Farber's disease), erythema induratum, lupus erythematosus profundus, periarteritis nodosa,

liquifying panniculitis associated with pancreatic disease, subcutaneous fat necrosis of the newborn, erythema nodosum, lipogranulomatosis subcutanea of Rothman-Makai and Weber-Christian syndrome.

In both our cases cultures from one of the subcutaneous nodules for bacteria, fungi, and acid fast bacilli were sterile. The antinuclear factor and lupus erythematosus cell preparation were negative, and the serum amylase and lipase levels were normal. Histological examination of the biopsy specimens showed no 'ghost cells' or enzymatic necrosis characteristic of liquifying panniculitis (Hughes, Apisarnthanarax & Mullins, 1975), no crystal formation or other features of subcutaneous fat necrosis of the newborn (Noojin, Pace & Davis, 1949), and no evidence of disseminated lipogranulomatosis (Zettrstrom, 1958). The polymorphonuclear cell infiltrate in the panniculus had a lobular rather than a septal pattern, and there was no pathological involvement of the dermis or epidermis. Vasculitis, a frequent finding in erythema nodosum (Winkelmann & Forstrom, 1975), was not seen.

Aetiology

The pathogenesis of Weber-Christian syndrome is still uncertain. Over the years many inciting agents have been proposed (Hallahan & Klein, 1951) including trauma (Fraser, 1942), halogen ingestion (Weber, 1925; Rosenberg & Cohen, 1942), bacterial (Sanford *et al.*, 1952; Carol *et al.*, 1941) or viral infections (Ostergaard, 1970), impaired fat metabolism (Koster & Behr, 1937), alpha-1-antitrypsin deficiency (Warter *et al.*, 1972), and autoimmune disorders (Neuman & Hewitt, 1964). It is likely that a heterogeneous group of diseases with different pathogenic mechanisms have been classified under the term 'Weber-Christian syndrome'. The development of specific laboratory tests may help differentiate these disorders.

In the present study the onset of Weber-Christian syndrome after an immunization in Case 1 suggests that trauma and stimulation of the immune system may have initiated the pathological process. In Case 2 it is interesting to note that the child did not develop skin lesions during the time he was lymphocyte-depleted after five exchange transfusions. With the onset of a possible transient graft-versus-host reaction a new crop of subcutaneous nodules appeared. On another occasion when the child was severely malnourished and T-lymphocytopenic he had the longest remission of his disease to date. The onset of fever associated with subcutaneous nodules heralded the return of his T-lymphocyte population to normal levels. This suggests that the T-lymphocyte may have an important role in the pathogenesis of Weber-Christian syndrome. The absence of immunoglobulins in the panniculus in one of our patients would be consistent with this concept.

Treatment

Many forms of therapy have been tried (Benson & Fowler, 1964) including antibiotics, antihistamines, antimalarials, multiple blood transfusions, X-ray therapy, hormones, vitamins, antituberculous drugs, sulphones, sulphapyridine, heavy metals, antirheumatics and corticosteroids. No therapeutic regimen, however, has produced consistent results. In our experience with two infants with Weber-Christian syndrome, prednisone effectively controlled the fever and toxicity associated with acute episodes of panniculitis, and in sufficient doses, prevented recurrences.

The ultimate prognosis, however, is still unknown. The panniculitis may spontaneously resolve after several years as mysteriously as it appeared (Lever, 1976). Unfortunately, the infants reported in the medical literature have continued to have episodes of Weber-Christian syndrome for as long as they were followed.

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REFERENCES

- ALBRECHTSEN, B. (1960) The Weber-Christian syndrome, with particular reference to etiology. *Acta dermatovenereologica*, **40**, 474.
- ARNOLD, H.A. & BAINBOROUGH, A.R. (1963) Weber-Christian disease with visceral involvement: case report and review of the literature. *Canadian Medical Association Journal*, **89**, 1138.
- BEERMAN, H. (1953) Weber-Christian syndrome. *American Journal of Medical Science*, **225**, 446.
- BENSON, R. & FOWLER, P.D. (1964) Treatment of Weber-Christian disease. *British Medical Journal*, **ii**, 615.
- BRILL, I.C. (1936) Relapsing febrile nonsuppurative panniculitis (Weber-Christian disease). In: *Medical Papers Dedicated to H.A.Christian*, pp. 694-704. Waverley Press Inc., Baltimore.
- CAROL, W.L.L., PRAKKEN, J.R. & ZWIJNDREGT, H.A. (1941) Erythema nodosum und 'Relapsing febrile nodular nonsuppurative panniculitis'. *Archiv für Dermatologie und Syphilis*, **182**, 329.
- CHRISTIAN, H.A. (1928) Relapsing febrile nodular nonsuppurative panniculitis. *Archives of Internal Medicine*, **42**, 338.
- CUMMINS, L.J. & LEVER, W.F. (1938) Relapsing febrile nodular nonsuppurative panniculitis (Weber-Christian disease). *Archives of Dermatology and Syphilology*, **38**, 415.
- DE GRACIANSKY, P. (1967) Weber-Christian syndrome of pancreatic origin. *British Journal of Dermatology*, **79**, 278.
- DELOR, C.J. & MARTZ, R.W. (1955) Weber-Christian disease with bone involvement. *Annals of Internal Medicine*, **43**, 591.
- FORSTROM, L. & WINKELMANN, R.K. (1975) Acute, generalized panniculitis with amylase and lipase in skin. *Archives of Dermatology*, **111**, 497.
- FRASER, J.F. (1942) Nonsuppurative panniculitis (Weber-Christian). *Archives of Dermatology and Syphilology*, **45**, 810.
- GILCHRIST, T.C. & KETRON, L.W. (1916) A unique case of atrophy of the fatty layer of the skin, preceded by the ingestion of the fat by large phagocytic cells: macrophages. *Bulletin of the Johns Hopkins Hospital*, **26**, 291.
- GOLDBERG, L.M. & RITZMANN, L.W. (1958) Unusual manifestations in a case of relapsing nodular febrile panniculitis (Weber-Christian disease). *American Journal of Medicine*, **23**, 788.
- HALLAHAN, J.D. & KLEIN, T. (1951) Relapsing febrile nodular nonsuppurative panniculitis (Weber-Christian disease); a review of literature and report of case. *Annals of Internal Medicine*, **34**, 1179.
- HUGHES, P.S.H., APISARNTHANARAX, P. & MULLINS, F. (1975) Subcutaneous fat necrosis associated with pancreatic disease. *Archives of Dermatology*, **111**, 506.
- KOSTER, L. & BEHR, E. (1937) Une forme speciale de la lipogranulomatose généralisée. *Acta Medica Scandinavica*, **93**, 30.
- KUNZER, W. *et al.* (1970) Verbrauchskoagulopathie bei Panniculitis nodularis febrilis non-suppurativa (Pfeifer-Weber-Christian). *Klinische Wochenschrift*, **48**, 193.
- LEVER, W.F. (1976) Personal communication, 23 January 1976.
- LEVER, W.F. & LEVER, G.S. (1975) Inflammatory diseases of the subcutaneous fat. In: *Histopathology of the Skin*, Chpt. 13, 5th edn. J.B.Lippincott Company, Philadelphia, U.S.A.
- MASICOWA, I. & WOJCIECHOWSKA, H. (1971) Zespół Webera-Christiana u 18-miesięcznego dziecka. *Polski Tygodnik Lekarski*, **26**, 1546.
- MAYER, M.M. (1973) The complement system. *Scientific American*, **229**, 54.
- MILNER, R.D.G. & MITCHINSON, M.J. (1965) Systemic Weber-Christian disease. *Journal of Clinical Pathology*, **18**, 150.
- NEUMAN, A. & HEWITT, J. (1964) La maladie de Weber-Christian est-elle une collagénose? A propos d'un cas. *Semaine des hôpitaux de Paris*, **40**, 1126.
- NOOJIN, R.O., PACE, B.F. & DAVIS, H.G. (1949) Subcutaneous fat necrosis of the newborn: certain etiologic considerations. *Journal of Investigative Dermatology*, **12**, 331.
- OSTERGAARD, P.A. (1970) Weber-Christian disease—Toxoplasmosis as a possible aetiological factor in panniculitis of Weber-Christian type. *Danish Medical Bulletin*, **17**, 158.
- PFEIFER, V. (1892) Über einen Fall von herdweiser Atrophie des subcutanen Fettgewebes. *Deutsches Archiv für Klinische Medizin*, **50**, 438.

- PILLEMAR, L. *et al.* (1954) The properdin system and immunity. I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science*, **120**, 279.
- REED, R.J., CLARK, W.H. & MIHM, M.C. (1973) Disorders of the panniculus adiposus. *Human Pathology*, **4**, 219.
- ROSENBERG, W.A. & COHEN, T.M. (1942) Relapsing, febrile, nodular, nonsuppurative panniculitis (Weber-Christian disease). *Illinois Medical Journal*, **81**, 59.
- SANFORD, H.N., EUBANK, D.F. & STENN, F. (1952) Chronic panniculitis with leucopenia (Weber-Christian syndrome). *A.M.A. American Journal of Diseases of Children*, **83**, 156.
- SCHOEN, I., REINGOLD, I.M. & MEISTER, L. (1958) Relapsing nodular nonsuppurative panniculitis with lung involvement: clinical and autopsy findings, with notes on pathogenesis. *Annals of Internal Medicine*, **49**, 687.
- TILDEN, I.L., GOTSCHALK, H.C. & AVAKIAN, E.V. (1940) Relapsing febrile, nonsuppurative panniculitis. *Archives of Dermatology and Syphilology*, **41**, 681.
- VESTERMARK, S. (1966) Weber-Christian syndrome in a newborn. *Acta Paediatrica Scandinavica*, **55**, 432.
- WARTER, J. *et al.* (1972) Syndrome de Weber-Christian associé à un déficit en alpha-1-antitrypsine. Enquête familiale. *Annales de Médecine Interne*, **123**, 877.
- WEBER, F.P. (1925) A case of relapsing nonsuppurative nodular panniculitis showing phagocytosis of subcutaneous fat-cells by macrophages. *British Journal of Dermatology*, **37**, 301.
- WINKELMANN, R.K. & FORSTROM, L. (1975) New observations in the histopathology of erythema nodosum. *Journal of Investigative Dermatology*, **65**, 441.
- ZETTERSTROM, R. (1958) Disseminated lipogranulomatosis (Farber's disease). *Acta Paediatrica Scandinavica*, **47**, 501.

Subungual exostoses of the fingers. Are they really uncommon?

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SUMMARY

Subungual exostoses on the fingers are usually considered rare. Six cases are reported and the literature reviewed.

Subungual exostoses are not uncommon on the great toe but are said to be rare on the fingers.

Hutchinson (1857) appears to have been the first to report the condition when he recalled seeing a young woman with an exostosis on her 'thimble finger'. Stocks & Barrington (1925) in a complete review of the literature on exostoses found only nine subungual exostoses on the fingers. Mason (1937) in a comprehensive treatise on tumours of the hand considered that less than a dozen of these lesions had ever been recorded. More recently Mangini (1967) reported 152 skeletal tumours in the hand but none was a subungual exostosis.

An extensive search of the literature has revealed only five further cases reported in 40 years (Table 1).

Six patients have been seen in recent years at two orthopaedic departments suggesting that subungual exostoses occur on the fingers more commonly than the paucity of reports suggests. The fact that half were referred after misdiagnosis and failed treatment indicates that some clinicians are unfamiliar with the condition and the ease with which it is distinguished radiographically from other subungual lesions. For this reason these patients are added to those already in the literature to clarify the clinical picture.

PRESENT SERIES

There were five females and one male. Their ages at the time the subungual exostosis was first noticed ranged from birth to 40 years, with a mean of 16 years, but they did not seek expert advice for 6 months to 20 years after this.

All patients presented with a solitary tender nodule protruding beneath the tip of the nail (Fig. 1) or under the edge, usually on the radial side. Two had marked deformity of the nail. Occurring pre-

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TABLE I

Author	Thumb	Index	Middle	Ring	Little	Not identified
Stocks & Barrington (1925)	2	3	1	1		2
Lowenthal (1964)		1 (4)*				
Evison & Price (1966)		1 (13)			1 (34)	
Jacobson (1971)	2					
Present series	3 (15) (13) (13)	2 (40) (15)	1 (Birth)			
Total	7	7	2	1	1	2

* Patients' ages, where known, in parentheses.

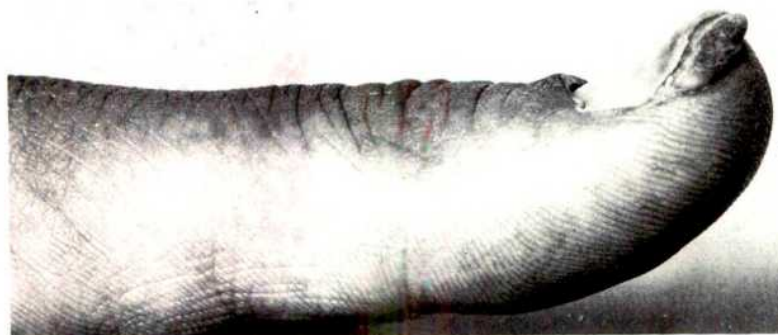


FIGURE 1.

dominantly on the thumb and index finger (Table I) three had recently increased in size and become painful if knocked or used in a repetitive activity, e.g. typing. None gave a history of significant trauma.

Radiographs showed a cancellous outgrowth from the dorsum of a normally shaped terminal phalanx, the trabeculae being continuous with the tuft. The tip of the exostosis unlike the margins was not always well defined, often presenting a ragged appearance merging into the soft tissue (Fig. 2).

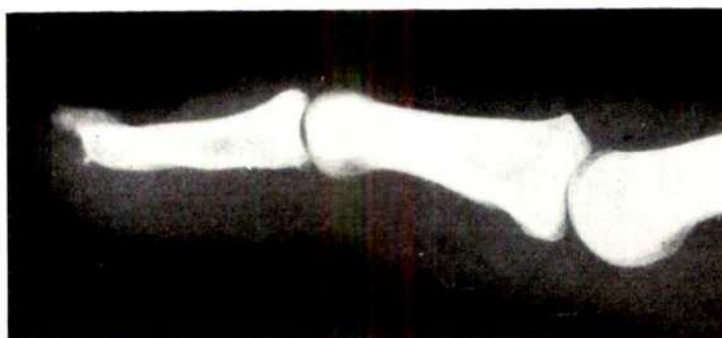


FIGURE 2.

Three patients were referred from other departments after failure of curettage under local anaesthesia. None had been X-rayed. In all three the exostosis recurred and one became infected.

In five patients surgical excision after avulsion of the nail and careful reflection of the nail bed and matrix was curative. One patient with gross nail deformity had the nail bed excised and skin grafted with no recurrence of the tumour or regeneration of the nail.

DISCUSSION

Subungual exostoses on the hand occur predominantly in the second and third decades and most are on the index finger and thumb. There is a preponderance of females to males in the ratio 2:1. This age, sex, and site incidence is similar to those exostoses occurring on the toes.

Radiography distinguishes them from other subungual lesions such as fibroma, granuloma pyogenicum, onychomycosis, verruca, glomus tumour, melanoma and epithelioma.

The aetiology remains obscure; trauma, inflammation, heredity and congenital dichotomy of the terminal phalanx have all been implicated without direct evidence. The present series does not confirm any of these theories.

Evison & Price (1966) suggested that they are not tumours but 'represent an ossifying form of fibrocartilaginous metaplasia arising in response to chronic stimulation'.

Those who recognize them consider them a relatively inconsequential lesion easily cured by simple excision and this probably accounts for their rarity in the literature.

With an increased awareness of the clinical features and by the simple expedient of a lateral radiograph of subungual lesions, clinicians may well save the patient further discomfort and themselves embarrassment.

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REFERENCES

- EVISON, G. & PRICE, C.H.G. (1966) Subungual exostosis. *British Journal of Radiology*, **39**, 451.
STOCKS, P. & BARRINGTON, A. (1925) *Eugenics Laboratory Memoirs*, **xxii**, Vol. 3, pt. 1, 69.
LOWENTHAL, K. (1964) *New York State Journal of Medicine*, **64**, 2691.
JACOBSON, S. (1971) *The Comparative Pathology of the Tumours of Bone*, p.94 Charles C. Thomas, Springfield, Illinois.
MASON, M.L. (1937) Tumours of the hand. *Surgery, Gynecology and Obstetrics*, **64**, 129.
MANGINI, U. (1967). Tumours of the skeleton of the hand. *Bulletin of the Hospital for Joint Diseases*, **28**, 61.
HUTCHINSON, J. (1857) *Lancet*, **ii**, 246.

Fabry's disease: biochemical and histochemical studies on hair roots for carrier detection

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SUMMARY

A method to assay α -galactosidase and acid phosphatase activities in single hair roots is described. Enzyme histochemical studies show that the distribution of acid phosphatase in the human hair root matches that of α -galactosidase. Histochemically, the main activity is located in the upper part of the sheath near the orifice of the duct of the sebaceous gland. This is confirmed by enzyme assays on different parts of the hair root after dissection. The variation in the values found in individual hair roots is improved by relating α -galactosidase to acid phosphatase activities. Storage experiments indicate a remarkable stability of both α -galactosidase and acid phosphatase in human hair roots.

Fabry's disease is caused by a ceramide trihexosidase deficiency. The ceramide trihexosidase involved is an acid α -galactosidase. Deficiency of this enzyme causes an accumulation of ceramide trihexosides and, to a certain extent, of other glycolipids within the lysosomes in many tissues (Sweeley & Klionsky, 1963). Fabry's disease can, therefore, be classified as a lysosomal storage disease.

A method for heterozygote detection in Fabry's disease using hair roots has recently been described by Grimm, Wienker & Ropers (1976). These authors demonstrated that the hair root is a suitable organ for heterozygote detection in this X-linked disorder. However, the method employed is both time-consuming and complicated. To correct for different hair root sizes they assayed both α -galactosidase activity and the protein content of single hair roots. For protein determination overnight staining followed by destaining (6 h) and scanning of a gel is involved, while for enzyme activity determination a photograph of the gel has to be scanned.

In this paper an easy and rapid method to measure α -galactosidase and acid phosphatase activities in a single hair root is described. Histochemical studies are reported showing that acid phosphatase is a suitable reference enzyme with respect to α -galactosidase, thus obviating protein estimation in single hair roots. After dissecting the hair root, α -galactosidase and acid phosphatase activities were assayed in different parts of the hair root and compared with the histochemically demonstrated enzyme distribution.

MATERIALS AND METHODS

Enzyme assays on single hair roots

Human hair roots were plucked from several areas of the scalp, and those with visible bulb and sheath were cut off just above the sheath and immediately immersed in 60 μ l distilled water containing 0.1% bovine serum albumin. After freezing (-20°C) and thawing 5 times, aliquots were taken for the enzyme assays.

α -Galactosidase: the reaction was started by the addition of 20 μ l of the hair root lysate to 20 μ l 14 mmol/l 4-methylumbelliferyl- α -D-galactoside in 0.2 mol/l citrate-phosphate buffer pH 4.6 (freshly prepared by heating to 40°C followed by sonification to dissolve the substrate).

The reaction mixture was incubated for 4 h at 37°C .

Acid phosphatase. 20 μ l of the hair root lysate was added to 20 μ l 25 mmol/l 4-methylumbelliferyl phosphate in 0.3 mol/l citrate-phosphate buffer pH 5.0. Due to the acidity of 4-methylumbelliferyl phosphate the final pH was 4.7. The reaction was allowed to proceed for 30 min at 37°C .

Both reactions were terminated by the addition of 400 μ l 0.1 mol/l ethylene diamine buffer, pH 11.4. The fluorescence was measured in a MPF-4 Perkin-Elmer fluorescence spectrophotometer at 445 nm after excitation at 360 nm.

Enzyme assays on dissected hair roots

Freshly obtained human hair roots were dissected into 4 parts by cutting them through the middle of the bulb, just below the sheath, through the middle of the sheath, and just above the sheath. The 4 parts of the hair root are referred to as matrix, upper bulb, lower sheath and upper sheath. The α -galactosidase and acid phosphatase activities in these sections of the human hair root were measured by the method described above.

Enzyme histochemistry on human hair roots and monkey skin biopsies

Human hair roots were plucked from the scalp, oriented for longitudinal sectioning in a saturated aqueous solution of methyl-carboxycellulose, and chilled with CO_2 gas.

Skin biopsies were taken from the scalp of a monkey (*Macaca mulatta*) and frozen in isopentane by chilling with a methanol-solid CO_2 mixture (-70°C). The skin biopsies were stored in a freezer at -96°C until use.

Sections were cut at 10 μm with a Walter Dittes cryostat (-25°C), taking care that longitudinal sections of human or monkey hair roots were obtained.

α -Galactosidase activity was detected using the semipermeable membrane method described by Lojda, Gossrau & Schiebler (1976). The sections were incubated for 2 h at 37°C , and mounted in glycerin-gelatin.

Acid phosphatase activity was demonstrated histochemically by the method of Meyer (1972). After 1 h incubation at 37°C , 2% glutaraldehyde in cacodylate buffer (0.4 mol/l, pH 7.3) was used for postfixation, haematoxylin for counterstaining (15 min), and Euparal for mounting the slices.

Storage experiment

Hair follicles obtained from one person were kept at room temperature without any special precaution. At intervals enzyme assays were performed during a period of 21 days.

RESULTS

The α -galactosidase activity in the hair roots of man and monkey was found to be restricted histo-

chemically to the inner part of the outer sheath in the area surrounding the opening of the duct of the sebaceous gland (see Fig. 1b, c and d).

Acid phosphatase activity is located in the papilla, as can be observed in monkey skin biopsy preparations (Fig. 1e and f). In addition, acid phosphatase activity is histochemically detected in the lower region of the cortex of the hair up to the site of transition of the matrix into the keratinized hair cuticle, but the main enzyme activity is found in the inner part of the external sheath both in man and monkey (Fig. 1a, e and f). In the external sheath especially marked acid phosphatase activity is observed near the opening of the duct of the sebaceous gland.

Dissection of the hair root followed by biochemical enzyme assay confirms that the activities of both enzymes are mainly located in the upper region of the hair follicle. Table 1 presents the relative activities of the 2 enzymes in 4 sections of the human hair root. The upper sheath contains 54% and 79% of the α -galactosidase and acid phosphatase activities, respectively.

In Table 2 the results of the measurements on 121 individual hair roots taken from 8 healthy persons are summarized. Although the extreme ranges for both α -galactosidase and acid phosphatase activities were rather wide (highest observed values/lowest observed values of 9.1 and 17.1, respectively), the corresponding range for the ratios of these two enzymes was appreciably narrower (highest observed value/lowest observed value of 5.8).

The effect of storing human hair follicles at room temperature for 21 days is shown in Table 3.

DISCUSSION

The hair root constitutes a complicated organ consisting of different sub-structures and cell types. Consequently, enzyme activities are not necessarily distributed equally over the whole hair follicle, but may be located in defined parts of it. If different enzymes are to be assayed in single hair roots, and to be expressed in relation to one another in order to correct for differences in hair root sizes, both enzymes should preferably have the same distribution because there is no guarantee that all sub-structures are present in any individual hair root obtained by plucking.

The present enzyme histochemical and biochemical studies show that the main activity of both α -galactosidase and acid phosphatase is located in the upper part of the sheath near the orifice of the sebaceous gland duct. In fact, acid phosphatase was selected as a reference enzyme for α -galactosidase, since histochemical studies of several other enzymes (Vermorken, in preparation) revealed that the distribution of acid phosphatase matched most closely that of α -galactosidase.

As a consequence of X-chromosome inactivation (Lyon, 1961) in a female heterozygous of Fabry's disease a mosaicism of α -galactosidase positive and negative cells can be expected. Due to the clonal origin of human hair follicles this mosaicism can be demonstrated by hair root analysis in X-linked disorders (Gartler *et al.*, 1971; de Bruyn, Oei & ter Haar, 1974).

The ranges found after measurements on 121 individual normal hair roots (Table 3) show that there is considerably more variation in acid phosphatase activity (highest value/lowest value ratio 17.1), than in α -galactosidase activity (highest value/lowest value ratio 9.1). Nevertheless, the variation in α -galactosidase/acid phosphatase activity is rather lower (highest value/lowest value ratio 5.8). For the purpose of carrier detection by means of hair root analysis in Fabry's disease, it is desirable to have a range of normal values as delimited as possible.

The results of the storage experiment (Table 3) indicate an amazing stability of α -galactosidase and acid phosphatase in human hair roots. This is in agreement with the findings of Hösli *et al.* (1977) with hexosaminidase A and B, and opens up interesting possibilities for the mailing of hair roots for diagnostic and screening purposes.

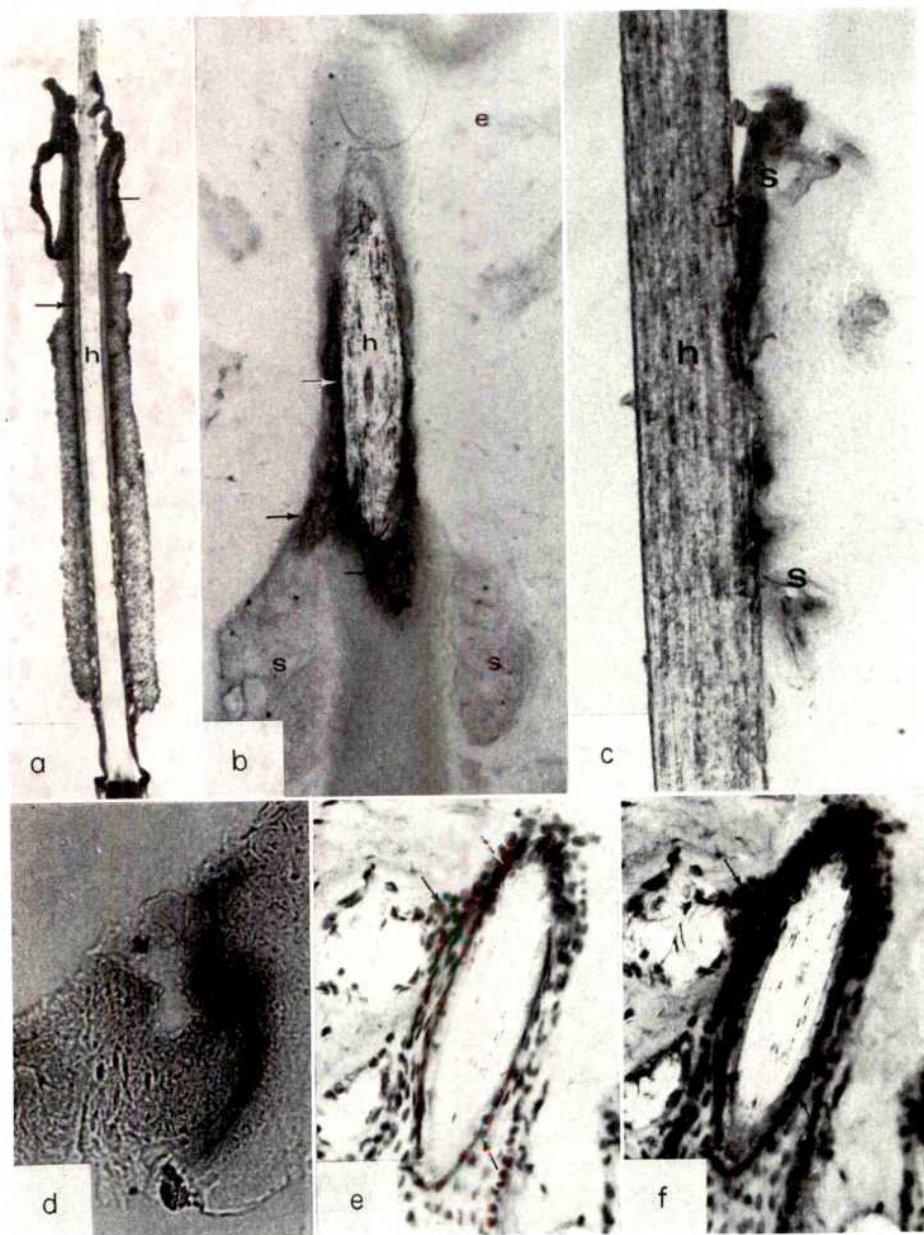


FIGURE 1. (a) Human hair ($\times 175$). Localization of acid phosphatase. h, Hair shaft; the arrows indicate the sites of acid phosphatase activity. (b) Skin of the scalp of a monkey ($\times 750$). h, Hair shaft; s, sebaceous gland; e, epidermis; the arrows indicate the sites of α -galactosidase activity. (c) Human hair ($\times 750$). Localization of α -galactosidase. h, Hair shaft, s, excretory duct of sebaceous gland. (d) Human hair ($\times 1750$). Localization of α -galactosidase. Detail of the opening of the excretory duct (see arrow). (e and f) Skin of the scalp of a monkey ($\times 850$). The arrow indicates the sites of acid phosphatase activity. Same area; (e) interference filter adjusted to transmit the red light fraction. (f) Filter adjusted to absorb and so accentuate the location of the red reaction product.

TABLE 1. Relative amounts of enzyme activity in different parts of microdissected hair roots

	Acid phosphatase (%)	α -Galactosidase (%)
Matrix	3	7
Upper bulb	6	11
Lower sheath	12	28
Upper sheath	79	54

TABLE 2. Results of the measurements on 121 hair roots from 8 persons

Control	Sex	No. of hair roots	α -Galactosidase*		Acid phosphatase*		α -Galactosidase	
							Acid phosphatase	
			Mean	s.d.	Mean	s.d.	Mean	s.d.
1	F	20	0.31	0.12	9.0	3.0	0.034	0.008
2	F	19	0.42	0.16	16.2	6.2	0.026	0.005
3	F	20	0.38	0.14	9.1	4.3	0.042	0.009
4	M	10	0.52	0.27	22.0	9.3	0.023	0.008
5	M	11	0.27	0.11	10.4	4.0	0.028	0.008
6	M	20	0.53	0.14	17.7	5.1	0.031	0.007
7	M	10	0.49	0.17	11.6	4.1	0.043	0.009
8	M	11	0.40	0.11	12.1	2.5	0.033	0.010

* Expressed in nmol/hair root/h.

TABLE 3. Effect of storing human hair roots at room temperature: On each day 10 individual hair roots obtained from one person were measured

Day	α -Galactosidase*		Acid phosphatase*		α -Galactosidase	
					Acid phosphatase	
	Mean	s.d.	Mean	s.d.	Mean	s.d.
0	0.54	0.17	17.3	4.7	0.032	0.007
1	0.51	0.18	12.5	4.6	0.042	0.009
4	0.45	0.09	15.0	2.3	0.034	0.006
7	0.58	0.11	15.0	3.5	0.040	0.010
14	0.42	0.14	11.2	1.8	0.037	0.009
21	0.34	0.11	5.8	0.6	0.055	0.017

* Expressed in nmol/hair root/h.

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REFERENCES

- DE BRUYN, C.H.M.M., OEI, T.L. & TER HAAR, B.G.A. (1974) Studies on hair roots for carrier detection in hypoxanthine-guanine phosphoribosyl transferase deficiency. *Clinical Genetics*, **5**, 449.
- GARTLER, S.M., SCOTT, R.C., GOLDSTEIN, J.L., CAMPBELL, B. & SPARKES, R. (1971) Lesch-Nyhan syndrome: rapid detection of heterozygotes by use of hair follicles. *Science*, **172**, 572.
- GRIMM, I., WIENKER, T.F. & ROPERS, H.H. (1976) Fabry's disease: heterozygote detection by hair root analysis. *Human Genetics*, **32**, 329.
- HÖSLI, P., SCHECK, L., AMSTERDAM, D. & VOLK, B.W. (1977) Hair roots in screening and diagnosis of Tay-Sachs disease. *Lancet*, **i**, 285.
- LOJDA, Z., GOSSRAU, R. & SCHIEBLER, T.H. (1976) *Enzym-histochemische Methoden*, 1. edn., p. 127, Springer-Verlag, Berlin.
- LYON, M.F. (1971) Gene action in the X-chromosome of the mouse. *Nature (London)*, **190**, 372.
- MEYER, A.E.F.H. (1972) Semi permeable membranes for improving the histochemical demonstration of enzyme activities in tissue sections. 1. Acid phosphatase. *Histochemie*, **30**, 31.
- SWEELEY, C.C. & KLIONSKY, B. (1963) Fabry's disease: classification as a sphingolipidosis and partial characterization of a novel glycolipid. *Journal of Biological Chemistry*, **238**, 3148.

Low nickel diet in the treatment of patients with chronic nickel dermatitis

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SUMMARY

Of 28 patients with chronic nickel dermatitis 17 experienced aggravation following oral ingestion of 2.5 mg nickel, but not a placebo tablet. The dermatitis of 9 of the 17 patients improved during a period of 6 weeks on a low nickel diet. The dermatitis of 7 of the 9 patients flared again when a normal diet was resumed.

Nickel excretion over a 24-h period was measured for 14 of the 17 patients by atomic absorption spectrophotometry before, during, and after the diet. Reduced excretion was seen during the diet with no statistically significant difference between patients whose dermatitis improved during the diet and those whose dermatitis showed no change.

In 1975 Christensen & Möller observed acute pompholyx in nickel sensitive patients following the ingestion of nickel in an amount comparable to that in a normal diet. Approximately 10% of the nickel in food is absorbed, while the remainder is excreted in the faeces (Horak & Sunderman, 1973). The absorbed nickel is excreted mainly in the urine and sweat (Hohnadel, 1973). Attempts have been made to correlate urinary excretion of nickel to oral ingestion of the metal (McNeely, Nechay & Sunderman, 1972). Menné & Thorboe (1976) found an increased amount of nickel in the urine of patients with nickel dermatitis during flares of the dermatitis.

In the current investigation we have attempted to determine whether recurrent pompholyx in nickel sensitive patients can be improved by a decrease in nickel ingestion and whether the excretion of nickel in urine will thereby be reduced.

MATERIALS AND METHODS

Twenty-six females and 2 males with hand eczema of dyshidrotic morphology, and with at least erythema and papules following patch test with 3.5% nickel sulphate in petrolatum, participated in the study. The duration of the nickel dermatitis was from 6 months to 17 years with a mean of 5.3

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years. Patients with more than one other positive patch test to substances in the standard series of the Finsen Institute were excluded.

A 24-h urine specimen was collected from each patient. A clinical examination was made, and close-up photographs of the hands and feet were taken. A blind challenge with tablets of identical appearance was carried out by giving each patient a placebo tablet and 3 days later a tablet containing 2.5 mg nickel as nickel sulphate. This sequence was chosen to prevent possible delay caused by a flare of the dermatitis following ingestion of nickel. Three days after ingestion of each tablet the patients were re-examined and comparative photographs were taken. These were enlarged to 1.5 times life size and evaluated blindly. The number of vesicles in the same areas before and after ingestion of the tablet was noted. Aggravation of the dermatitis was defined as increase of subjective symptoms such as itching combined with a greater number of vesicles or eruption of vesicles in areas not previously affected. Increased itching with no definite change in the number of vesicles was considered an equivocal reaction.

Only those patients who experienced definite or equivocal aggravation following ingestion of the nickel tablet, but not the placebo, continued the study. These patients were instructed to reduce nickel intake for 6 weeks by following the diet given in Table 1 and to avoid external contact with

TABLE 1. Diet instructions given to all patients*

Permitted foods	Prohibited foods
All meats	Canned foods and acid
Poultry	foods cooked in stainless
Fish, except herring	steel utensils
Eggs	
Milk	Herring
Yoghurt	Oysters
Butter	
Margarine	
Cheese	
1 medium-sized potato per day	Asparagus
Small amounts of the following:	Beans
Cauliflower	Mushrooms
Cabbage	Onions
Carrots	Corn (Maize)
Cucumber	Spinach
Lettuce	Tomatoes
	Peas
Polished rice	
Flour (except whole-grain)	Whole-grain flour
Fresh fruits, except pears	Fresh and cooked pears
Marmalade/jam	Rhubarb
Coffee	Tea
Wine	Cocoa and chocolate
Beer	
	Baking powder

* Compiled from Schroeder, Balassa & Tipton (1962), Dencker *et al.* (1971), Schlettwein-Gsell & Mommsen-Straub (1971) and Thomas *et al.* (1974).

nickel throughout the period of investigation. The patients kept diet diaries and recorded eruptions of the dermatitis. They were re-examined after 2, 4 and 6 weeks of dieting. The only treatment given while the patients were dieting was fluocortolone cream or a less potent corticosteroid cream. A 24-h urine specimen was collected 2 weeks or more after initiation of the diet. After 6 weeks of dieting the frequency and severity of eruptions of the dermatitis were compared with pre-diet activity. Based on these parameters it was decided whether or not improvement had occurred. The patients were then asked to resume their normal diets. Two to 4 weeks later they were re-examined, the severity of the dermatitis was noted, and a 24-h urine specimen was collected.

The urine specimens were collected in acid-washed containers. Nickel was extracted from the urine with diethylammonium-diethyldithiocarbamate in xylene following the addition of nitric acid (Suprapur, Merck) and a buffer. The amount was determined by atomic absorption spectrophotometry, and the detection level was found to be 0.2 parts per billion (10^9).

RESULTS

Thirteen patients had definite aggravation and 4 had equivocal reactions after ingestion of the nickel tablet, while none experienced aggravation following ingestion of the placebo. The eruptions usually occurred after 2 or 3 days, but 4 patients suffered urticaria-like eruptions which appeared 2–6 h after ingestion of the nickel tablet and lasted 3 or 4 days. Oedema of the fingers and face was seen in one patient.

During the diet period the dermatitis cleared in 3 and improved in 6 of the 17 patients. After resuming a normal diet 7 of the 9 patients whose dermatitis had improved or cleared experienced recurrence of the hand dermatitis to pre-diet severity. For 6 patients this recurrence was seen after less than 2 weeks, for 1 patient after 4 weeks.

Information regarding the amount of nickel in the urine before and during the diet period was available for 14 patients. Previous to ingestion of the nickel tablet, the 24-h nickel excretion varied from 0.42 to 3.6 μg with a mean of 1.47 μg . No correlation was found between the urinary excretion of nickel and the severity of the dermatitis at the initiation of the study or to the severity of the flare following nickel ingestion. In 11 of the 14 patients there was reduced excretion during the period of dieting (Figs 1 and 2). This reduction occurred both among patients whose dermatitis improved and those with no change in the dermatitis, and there was no statistically significant difference between these two groups ($P > 0.05$ by the Mann Whitney test). No correlation was found between the degree of reduction in nickel excretion and the degree of improvement following the diet period. The nickel excretion of 6 patients remained low after a normal diet was resumed, while the nickel excretion of 10 patients increased—for 6 patients to pre-diet levels (Figs 1 and 2).

DISCUSSION

The results presented here indicate that it is possible to reduce nickel ingestion and that nickel dermatitis of dyshidrotic morphology can be improved by a reduction in nickel intake. The improvement could be explained partially as spontaneous variations in disease activity. However, the aggravation shortly following return to a normal diet in 7 of the 9 patients whose dermatitis improved while dieting supports the contention that the improvement was, in fact, due to a reduction of nickel intake.

There was no statistically significant difference in the reduction in nickel excretion among patients whose dermatitis improved during the diet compared with those who showed no change in the dermatitis. Violation of the diet could account for the increase in nickel excretion in 3 of the patients. Other

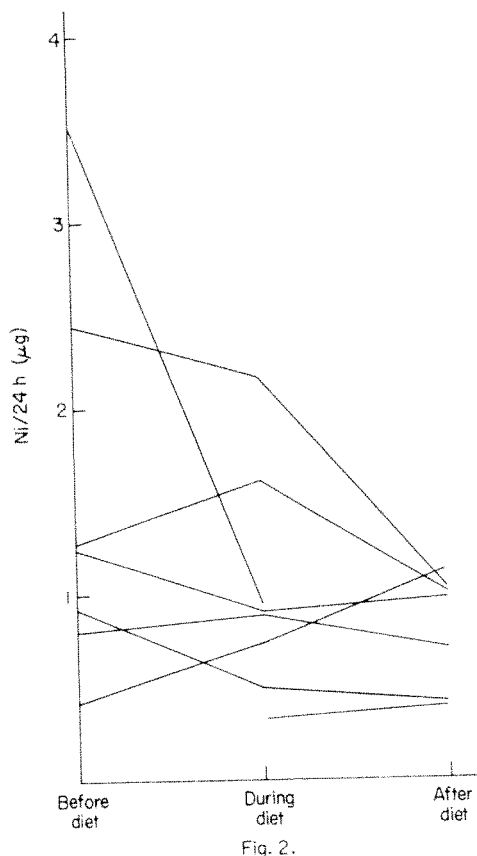
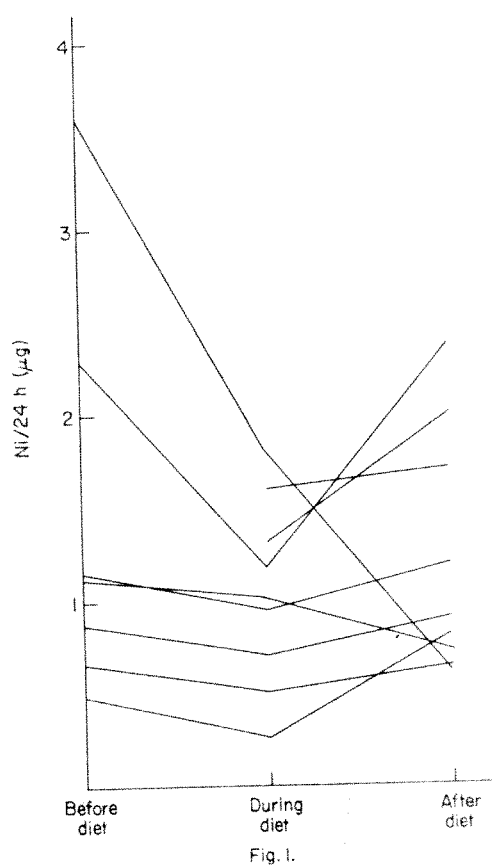


FIGURE 1. Urinary nickel excretion in 9 patients with clinical improvement.

FIGURE 2. Urinary nickel excretion in 8 patients without clinical improvement.

possible explanations of the dissociation between the clinical picture and/or nickel excretion could be individual variations in the degree of sensitization and continued external exposure to nickel.

In spite of the aggravation following resumption of a normal diet, the nickel excretion of 6 of 16 patients remained low (Figs 1 and 2). This could be due to depletion of the total body content of nickel as a result of dieting. After return to a normal diet the serum levels of nickel could quickly return to pre-diet levels. Nickel reserves would build up more slowly and low nickel excretion might be seen over a longer period.

A moderate strength corticosteroid cream was used for topical therapy throughout the period of investigation. This was chosen to alleviate the symptoms of the dermatitis without suppressing new eruptions and to ensure that the patients would not seek treatment elsewhere.

The daily ingestion of nickel in Sweden is estimated to be 0.76 mg (range 0.2–4.46 mg) (Dencker, Fristedt & Övrum, 1971) which is comparable to that in the United States (0.3–0.6 mg/day) (Committee on Environmental Pollutants, 1975). The nickel content of foods is partially determined by the components of the soil, and it is probable that increased industrialization has led to an increase in the amount of nickel ingested (Committee on Environmental Pollutants, 1975). With the exception of certain fish and other seafoods, nickel does not ordinarily accumulate in animal tissue. The nickel

content of canned foods has been shown to be significantly higher than that of fresh foods (Thomas, Roughan & Watters, 1974). This is probably due to leaching of nickel from the equipment used in processing canned foods. The low nickel diet (Table 1) was compiled on the basis of the nickel content of foods in European countries and the United States (Schroeder, Balassa & Tipton, 1962; Dencker *et al.*, 1971; Schlettwein-Gsell & Mommsen-Straub, 1971; Thomas *et al.*, 1974). To our knowledge the nickel content of Danish food has never been recorded. Both nickel content and normal daily consumption of each food item were taken into consideration in preparing the diet. It was felt that the low nickel diet represented no risk to the patient since it has not been convincingly demonstrated that nickel is an essential dietary ingredient (Committee on Environmental Pollutants, 1975).

It is concluded that for some patients with nickel dermatitis limitation of nickel ingestion is valuable in the management of the dermatitis. Similar experiments with longer diet periods and simultaneous determination of the serum levels of nickel would provide additional information on this subject. It was evident during this study, however, that it is difficult to maintain the proposed diet for a prolonged period. Nickel binding with chelating agents should therefore also be attempted.

REFERENCES

- CHRISTENSEN, O.B. & MÖLLER, H. (1975) External and internal exposure to the antigen in the hand eczema of nickel allergy. *Contact Dermatitis*, **1**, 136.
- COMMITTEE ON MEDICAL AND BIOLOGIC EFFECTS OF ENVIRONMENTAL POLLUTANTS (1975) *Nickel*. National Academy of Sciences, Washington, D.C.
- DENCKER, I., FRISTEDT, B. & ÖVRUM, P. (1971) Järn, koppar, krom, nickel, bly och kobolt i föda. *Läkartidningen*, **68**, 4027.
- HOHNADL, D.C., SUNDERMAN JR., F.W., NECHAY, M.W. & MCNEELY, M.D. (1973) Atomic absorption spectrometry of nickel, copper, zinc, and lead in sweat collected from healthy subjects during sauna bathing. *Clinical Chemistry*, **19**, 1288.
- HORAK, E. & SUNDERMAN, W. (1973) Fecal nickel excretion by healthy adults. *Clinical Chemistry*, **19**, 429.
- MCNEELY, M.D., NECHAY, M.W. & SUNDERMAN JR., F.W. (1972) Measurements of nickel in serum and urine as indices of environmental exposure to nickel. *Clinical Chemistry*, **18**, 992.
- MENNÉ, T. & THORBOE, A. (1976) Nickel dermatitis—nickel excretion. *Contact Dermatitis*, **2**, 353.
- SCHLETTWEIN-GSELL, D. & MOMMSEN-STRAUB, S. (1971) Spurenelemente in Lebensmitteln V Nickel. *International Zeitschrift für Vitaminforschung*, **41**, 429.
- SCHROEDER, H.A., BALASSA, J.J. & TIPTON, I.H. (1962) Abnormal trace metals in man—nickel. *Journal of Chronic Diseases*, **15**, 51.
- THOMAS, B., ROUGHAN, J.A. & WATTERS, E.D. (1974) Cobalt, chromium and nickel content of some vegetable foodstuffs. *Journal of the Science of Food and Agriculture*, **25**, 771.

Treatment of eczema with cyclophosphamide and azathioprine*

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SUMMARY

Nine patients with eczema were treated with the immunosuppressive drugs cyclophosphamide and azathioprine. In all patients the eczema was severe and incapacitating and had not responded satisfactorily to systemic and topical corticosteroid therapy. The eczema improved in all patients and systemic corticosteroids, which had resulted in undesirable side-effects, could be decreased or stopped. Leukopenia was not necessary for achieving clinical improvement. Several months of treatment were needed to obtain significant improvement in the eczema and long term remissions followed cessation of therapy.

Radiotherapy and corticosteroid treatment have proved to be invaluable in the management of severe eczema, but there remains a number of patients in whom these measures are not adequate. The side-effects of corticosteroids often limit their usefulness in patients requiring long-term treatment. There have been few references in the literature to the treatment of eczema with cytostatic drugs. Gubner, August & Ginsberg (1951) successfully treated one case of atopic eczema with aminopterin, a folic acid antagonist. Waldbott (1952) reported marked improvement in 4 patients with atopic eczema treated with nitrogen mustard. Eisen, Demis & Crosby (1962) had success in one case of atopic eczema treated intermittently for 5 months with thioguanine. Demis, Brown & Crosby (1964) treated 8 patients suffering from severe eczema with thioguanine for an average duration of 2 months with good results in 4. Lambert & Salmon (1966) treated a severe case of erythroderma for 8 months with azathioprine with remarkable improvement. Johansson & Juhlin (1970) treated 3 patients with atopic dermatitis and one with eczema with azathioprine for 2-4 weeks without benefit. We treated 9 patients suffering from severe eczema with cytostatic drugs. Six received cyclophosphamide, one azathioprine and two both drugs at different times.

PATIENTS AND METHODS

The age and sex of the 9 patients treated with cyclophosphamide and azathioprine are shown in Table 1. In the first 3 patients (Cases 1-3) the eczema was regarded as atopic, in 5 (Cases 4-8) as

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TABLE 1. Results of treatment of eczema with cyclophosphamide (C) and azathioprine (A)

Patient number	Age (years)	Sex	Previous duration of eczema (years)	Drug	Immunosuppressive treatment		% Skin involvement	
					Weekly dose (mg)	Duration (months)	Before	After
1	32	M	15	C	700-200	14	70	5
2	37	F	14	C	700-200	11½	90	2
3	56	M	2	C	700-500	6	100	5
4	60	M	1½	A	700	3	50	5
5	66	F	13	C	200-700	40	80	5
				A	87.5-350	6		
6	43	F	7	C	300-700	24+	90	10
7	63	M	¾	C	300-700	12	100	2
8	66	F	6	C	500	2	100	15
				A	350	1½		
9	53	F	6	C	700	8	80	0

seborrhoeic and Case 9 had disseminated varicose eczema. The previous duration of the eczema ranged from 9 months to 15 years (see Table 1). All patients except one had had one or more episodes of generalized exfoliative dermatitis. In this patient (Case 9) the eczema had been widespread for 3 years. All patients had had prolonged treatment with systemic corticosteroids with inadequate response. They were incapacitated and required frequent hospitalization or long periods away from work. Two patients (Cases 1 and 3) were Cushingoid in appearance at the start of immunosuppressive therapy. In addition patient 1 had severe peptic ulceration, difficult to control with medication. Three patients (Cases 2, 4 and 8) had marked atrophy of the skin due to prolonged corticosteroid treatment. Three patients had previously been treated with radiotherapy (Cases 1, 2 and 5) with no appreciable effect. Patient 5 had been treated elsewhere with 6-mercaptopurine and methotrexate with some improvement. Treatment with methotrexate and hydroxyurea in Case 4 resulted in initial improvement which was not maintained during a treatment period of 5½ months and he was referred to us for further management.

Cyclophosphamide was started orally in a dose of 100 mg daily which was reduced according to the total white cell count. A white cell count of 3000/mm³ was regarded as an indication to stop treatment but none of our patients reached this low level. The dose of cyclophosphamide was reduced initially according to the fall in white cell count and later to the clinical response. When the dose was reduced, the drug was administered on fewer consecutive days of the week so that there would be longer periods when the bladder mucosa was not exposed to alkyl radicals excreted in the urine. The tablets were administered in the morning and the patient was told to maintain a high fluid intake. Initially white cell counts were done at weekly intervals but once the tolerance to cyclophosphamide was established, counts were done once monthly.

Azathioprine was administered in a dose of 50 or 100 mg daily and thrombocyte counts were done initially at weekly intervals and later once a month. In no patient was the critical level of 100,000 thrombocytes per mm³ reached at which treatment would have been stopped. One patient (Case 4) was treated with azathioprine only as he had a history of previous bladder disease. The remaining 8 patients were treated with cyclophosphamide and 2 (Cases 5 and 8) were subsequently given azathioprine. The drugs were not given at the same time. Treatment with systemic and topical corticosteroids

was continued while the patients were receiving the cytostatic drugs, and decreased when improvement occurred.

RESULTS

In order to assess the results of treatment the degree of skin involvement was expressed as a percentage, 100% indicating generalized erythroderma with exfoliation. It is evident from Table 1 that the eczema decreased markedly during treatment in all patients. It was possible to decrease the dose of systemic corticosteroids in all patients and to stop them in 5 (Cases 2-4, 7 & 9). The duration of treatment with cyclophosphamide and azathioprine varied from 3 to 46 (average 14) months. The time taken to achieve significant improvement in the eczema varied from 1 to 7 (average 3.4) months. During treatment the white cell count fell in all patients, the lowest level being about 50% less than the initial. The average white cell count during treatment was in the region of 8000. The lowest total white cell count was 3800 in Case 2 after 6 months of treatment. There was no reduction in the number of platelets in the 3 patients who received azathioprine. No alopecia was noted in any of the patients during treatment. In Case 2, who had scalp involvement, hair growth improved as the eczema came under control. Significant improvement in the eczema was seen after an average treatment period of 3 months and maximal improvement after 6 months. There was no apparent relationship between the total white count and the clinical response. After treatment with the immunosuppressive drugs was stopped, remissions varied from 1 to 60 (average 24) months.

Special features during and after immunosuppressive therapy and side-effects encountered in each patient may be briefly summarized as follows:

Case 1. This patient was severely incapacitated by eczema and peptic ulceration and was Cushingoid in appearance at the start of treatment. He responded well to a 14 months course of cyclophosphamide, and systemic corticosteroids were decreased and stopped. For 7 months afterwards no systemic treatment was required but subsequent relapses required systemic corticosteroid treatment. Three and a half years after cyclophosphamide was stopped a severe relapse occurred. Azathioprine was given but had to be stopped after 2 weeks on account of severe nausea and diarrhoea. At the time of writing he was starting another course of cyclophosphamide. Azoospermia following the first course was apparently reversible as his wife had a baby 2½ years after it was discontinued (a second sperm count was refused).

Case 2. This patient had no side-effects and has required no systemic treatment in the 2 years since cyclophosphamide was stopped. She has had minor relapses of small areas of eczema easily controlled with ointments.

Case 3. This patient was given small intermittent doses of systemic corticosteroids in the 14 months after cyclophosphamide was stopped. Systemic treatment was actually unnecessary for the mild residual areas of desquamation but his general practitioner gave him tablets instead of ointment as the former are cheaper.

Case 4. This patient was given azathioprine rather than cyclophosphamide because of a history of previous bladder disease. After 3 months of treatment, when the eczema had almost completely cleared and systemic corticosteroids had been stopped, he was found to have a bladder carcinoma. The eczema was subsequently kept under control with local treatment only during a period of 5 years.

Case 5. This elderly woman was Cushingoid from long-term corticosteroid therapy. During 4 years of treatment with cyclophosphamide and azathioprine the eczema improved remarkably, but in the end both drugs were stopped because of side-effects (haematuria in the case of cyclophosphamide and severe nausea and vomiting from azathioprine). Within a month of stopping treatment the eczema relapsed.

Case 6. The patient who was erythrodermic at the start of treatment improved considerably during 2 years on intermittent courses of cyclophosphamide and corticosteroids. During the 2 years since she has stopped cyclophosphamide the condition has worsened by about 10%.

Case 7. The patient who was erythrodermic at the start of treatment with cyclophosphamide, was almost free of eczema when it was stopped 12 months later. In 20 subsequent months without any systemic treatment there has been no deterioration in his condition.

Case 8. At the start of treatment this elderly woman was erythrodermic and had marked steroid atrophy of the skin. The eczema improved considerably during treatment but both cyclophosphamide and azathioprine had to be stopped because of severe gastro-intestinal side-effects after 8 and 6 weeks respectively. In the following 18 months the eczema has remained a problem on her hands in spite of treatment with radiotherapy and systemic corticosteroids.

Case 9. During 8 months of treatment with cyclophosphamide this patient's eczema improved considerably and was almost completely under control when the drug was stopped because of an *E. coli* cystitis. She has had no further attacks in the past 18 months.

DISCUSSION

Disturbances in humoral and cell mediated immunity occur in atopic dermatitis (Hanifin & Lobitz, 1974). Both cyclophosphamide and azathioprine have been shown to suppress antibody production (Berenbaum & Brown, 1964; Levy *et al.*, 1972) and to have an anti-inflammatory effect (Hurd, 1973). In patients with atopic eczema there is a significant decrease in the numbers of circulating T lymphocytes with a resultant decrease in cell mediated immunity (Luckasen *et al.*, 1974; Carapeto *et al.*, 1976). Cyclophosphamide by suppressing B cell activity causes an enhancement in delayed hypersensitivity (Horwitz, 1974; Kerckhaert, Van den Berg & Hofhuis, 1974; Askenase, Hayden & Gershon, 1975).

In treating patients with azathioprine for diseases assumed to have an immunological basis Swanson & Schwartz (1967) found that leucopenia was not necessary for immunosuppression. Similar findings were reported in patients treated with cyclophosphamide for rheumatoid arthritis and systemic lupus erythematosus (Co-operating Clinics Committee of the American Rheumatism Association, 1970; Horwitz, 1974). This is in accord with our experience that clinical improvement in eczema can be obtained in the absence of leucopenia. Cyclophosphamide is easier to administer than azathioprine as it seldom has a thrombocytopenic action in therapeutic doses and regular platelet counts are not necessary (Hall, 1967).

In our patients the average duration of treatment required before a marked improvement occurred was $3\frac{1}{4}$ months. The poor results obtained by Johansson & Juhlin (1974) in the treatment of atopic dermatitis and of eczema with azathioprine may have been due to the short treatment period (4 weeks and less). In other diseases long treatment periods with cytostatic drugs have been found necessary to obtain improvement (Fosdick, Parsons & Hill, 1968; Schubert *et al.*, 1970; Moncrieff *et al.*, 1969;

Lambert & Salmon, 1966). In 6 of our patients long-term remissions followed cessation of cytostatic treatment, which had previously been impossible to achieve with long-term systemic corticosteroid therapy.

We have found the immunosuppressive drugs cyclophosphamide and azathioprine to be of considerable value in the treatment of severe intractable eczema although the significance of immunological disturbances in eczema and the mode of action of these drugs is still unknown.

REFERENCES

- ASKENASE, P.W., HAYDEN, B.J. & GERSHON, R.K. (1975) Augmentation of delayed-type hypersensitivity by doses of cyclophosphamide which do not affect antibody responses. *Journal of Experimental Medicine*, **141**, 697.
- BERENBAUM, M.C. & BROWN, I.N. (1964) Dose-response relationships for agents inhibiting the immune response. *Immunology*, **7**, 65.
- CARAPETO, F.J., WINKELMANN, R.K. & JORDON, R.E. (1976) T and B lymphocytes in contact and atopic dermatitis. *Archives of Dermatology*, **112**, 1095.
- CO-OPERATING CLINICS COMMITTEE OF THE AMERICAN RHEUMATISM ASSOCIATION (1970) A controlled trial of cyclophosphamide in rheumatoid arthritis. *New England Journal of Medicine*, **283**, 883.
- DEMIS, D.J., BROWN, C.S. & CROSBY, W.H. (1964) Thioguanine in the treatment of certain autoimmune, immunologic and related diseases. *American Journal of Medicine*, **37**, 195.
- EISEN, B., DEMIS, D.J. & CROSBY, W.H. (1962) Thioguanine therapy. Systemic lupus erythematosus, atopic dermatitis and other nonmalignant diseases. *Journal of the American Medical Association*, **179**, 789.
- FOSDICK, W.M., PARSONS, J.L. & HILL, D.F. (1968) Preliminary report: Long-term cyclophosphamide therapy in rheumatoid arthritis. *Arthritis and Rheumatism*, **11**, 151.
- GUBNER, R., AUGUST, S. & GINSBERG, V. (1951) Therapeutic suppression of tissue reactivity. II. Effect of aminopterin in rheumatoid arthritis and psoriasis. *American Journal of the Medical Sciences*, **221**, 176.
- HALL, T.C. (1967) Pharmacologic studies of cyclophosphamide (NSC-26271) and its congener phosphoramide mustard (NSC-69945). *Cancer Chemotherapy Reports*, **51**, 335.
- HANIFIN, J.M. & LOBITZ, W.C. (1977) Newer concepts of atopic dermatitis. *Archives of Dermatology*, **113**, 663.
- HORWITZ, D.A. (1974) Selective depletion of Ig-bearing lymphocytes by cyclophosphamide in rheumatoid arthritis and systemic lupus erythematosus. Guidelines for dosage. *Arthritis and Rheumatism*, **17**, 363.
- HURD, E.R. (1973) Immunosuppressive and anti-inflammatory properties of cyclophosphamide, azathioprine and methotrexate. *Arthritis and Rheumatism*, **16**, 84.
- JOHANSSON, S.G.O. & JUHLIN, L. (1970) Immunoglobulin E in 'healed' atopic dermatitis and after treatment with corticosteroids and azathioprine. *British Journal of Dermatology*, **82**, 10.
- KERCKHAERT, J.A., VAN DEN BERG, G.J. & HOFHUIS, F.M.A. (1974) Influence of cyclophosphamide on the delayed hypersensitivity in the mouse after immunization with histocompatibility antigen. *Journal of Immunology*, **113**, 1801.
- LAMBERT, P.H. & SALMON, J. (1966) Traitement immuno-suppressif chez 15 patients atteints d'affections auto-immunes ou de maladies ayant une pathogenie immunologique. *Acta clinica Belgica*, **21**, 171.
- LEVY, J., BARNETT, E.V., MACDONALD, N.S., KLINENBERG, J.R. & PEARSON, C.M. (1972) The effect of azathioprine on gammaglobulin synthesis in man. *Journal of Clinical Investigation*, **51**, 2233.
- LUCKASEN, J.R., SABAD, A., GOLTZ, R.W. & KERSEY, J.H. (1974) T and B lymphocytes in atopic eczema. *Archives of Dermatology*, **110**, 375.
- MONCRIEFF, M.W., WHITE, R.H.R., OGG, C.S. & CAMERON, J.S. (1969) Cyclophosphamide therapy in the nephrotic syndrome of childhood. *British Medical Journal*, **1**, 666.
- SCHUBERT, J.C.F., SCHUBERT, H., HOLTZ, G. & MARTIN, H. (1970) Immunosuppressive Langzeittherapie des Lupus erythematoses visceralis. *Deutsche Medizinische Wochenschrift*, **95**, 1768.
- SWANSON, M.A. & SCHWARTZ, R.S. (1967) Immunosuppressive therapy. The relation between clinical response and immunologic competence. *New England Journal of Medicine*, **277**, 163.
- WALDBOTT, G.L. (1952) Nitrogen mustard in the treatment of bronchial asthma. *Annals of Allergy*, **10**, 428.

An animal model for evaluating the local and systemic effects of topically applied corticosteroids on epidermal DNA synthesis

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SUMMARY

A simple animal model for studying topically applied glucocorticosteroids for their effect on epidermal DNA synthesis is described. It is designed so that their potential for systemic side effects may be predicted and its usefulness in this respect is illustrated by the results obtained with two of the steroids investigated. One, a new formulation Temetex, is predicted to have considerable systemic potential. The other, Molivate, is remarkable for having moderate local, without demonstrable systemic activity.

An animal model assay system for the evaluation of the epidermal antimitotic activity of topically applied drugs has already been described (du Vivier *et al.*, 1976). It has now been used to assess the comparative actions of a series of topically applied corticosteroid formulations on DNA synthesis. Both local activity and potential for systemic toxicity may be determined simultaneously.

MATERIALS AND METHODS

Animals

Hairless mice (strain A2hr), random bred, 2-4-months-old and of either sex were used. Animals were dosed in three groups of six, two of the groups with steroid formulations and one group with Cream E45 as control.

Formulations

- (i) 1% Hydrocortisone cream (Efcortelan cream: Glaxo).
- (ii) 0.01% Fluocinolone acetonide ointment (Synandone ointment: ICI).
- (iii) 0.025% Fluocinolone acetonide cream (Synalar cream: ICI).
- (iv) 0.05% Clobetasone butyrate cream (Molivate cream: Glaxo).
- (v) 0.1% Betamethasone 17-valerate cream (Betnovate cream: Glaxo).

- (vi) 0.1% Diflucortolone valerate cream (Temetex cream: Roche).
- (vii) 0.05% Clobetasol propionate cream (Dermovate cream: Glaxo).
- (viii) 0.05% Diflorasone diacetate cream (Florone: Upjohn).
- (ix) Control—white soft paraffin 14.5%, light liquid paraffin 11.6%, anhydrous lanolin 1% in a specially formulated emulsion base (Cream E45: Boots).
- (x) Base for clobetasone butyrate cream (Molivate base: Glaxo).

Dosage

0.03 ml of the test drug formulation was applied, and spread over 3 cm² of the anterior dorsal skin (hereafter referred to as the neck) with a seeker for 60 s. The posterior half of the dorsal surface of the animal (hereafter referred to as the back) was left untreated.

All animals were injected subcutaneously with 25 μ Ci (methyl-³H) thymidine, specific activity 5 Ci/mmol (The Radiochemical Centre, Amersham), in the right thigh 1 h before being killed.

Animals were killed by ether inhalation 6 or 24 h after the application of the test formulation.

Preparation of epidermal samples

After death, the neck and back skin samples, with at least 2 cm of unsampled skin left between them (to avoid inclusion of treated skin in the back sample) were removed. The back sample was taken from the left side of the animal, well away from the site of thymidine injection.

The epidermis was separated from the dermis by placing the sample, dermis downwards, on a stainless steel plate at 57°C for 25 s. The epidermis could then be easily separated from the dermis with a scalpel blade. The epidermal samples were wrapped in aluminium foil and stored at -20°C until required for analysis.

Extraction of DNA

Approximately 30 mg of epidermis in 5 ml of a 0.24 M sodium phosphate buffer solution, containing 8 M urea, 1% sodium lauryl sulphate and 1 mM ethylenediaminetetraacetic acid (EDTA) at pH 6.8, was lysed using an ultrasonic disintegrator (Rapidis 180G: Ultrasonics) with a 3 mm probe. DNA was extracted from the cell lysate by column chromatography using hydroxyapatite (HAP). 50 ml Econocolumns (Bio-Rad Laboratories) were used. One g of HAP (Bio-Gel HTP: Bio-Rad Laboratories) was added to the columns and washed in with a starting buffer solution of 0.24 M sodium phosphate buffer containing 8 M urea at pH 6.8. The epidermal cell lysate was then added and washed through the HAP bed with 50 ml of starting buffer to remove RNA and protein. The urea was then removed with 50 ml of 0.14 M sodium phosphate buffer at pH 6.8. Double stranded DNA, which binds to the HAP under low salt buffer conditions (Meinke, Goldstein & Hall, 1974), was eluted with 0.48 M sodium phosphate buffer (high salt) at pH 6.8, and a 2 ml sample collected.

Estimation of DNA specific activity

The DNA concentration of the sample was determined by UV absorption at 260 nm in a spectrophotometer (Uvichem: Hilger and Watts). The purity of the sample was confirmed by the A₂₆₀:A₂₈₀ ratio (which should be approximately 2:1. If less than 1.8:1 the sample is assumed to be contaminated with RNA and/or protein, indicating inadequate washing prior to elution of DNA). The concentration of DNA in the sample can be calculated on the basis that one optical density is approximately equal to 50 μ g DNA/ml (Felsenfeld & Hirschman, 1965).

0.5 ml aliquots of the sample were added to 12 ml scintillation cocktail (3 parts PCS (Searle): 2 parts toluene) and 0.2 ml N HCl in scintillation vials. Each sample was prepared in triplicate and counted for 20 min/vial in a scintillation counter (Packard Tri-Carb 3358). The results were initially

expressed as the mean counts per minute (c.p.m.) for the vial triplets. The efficiency of counting for each sample was determined from the relevant channels ratio for that sample using a counting efficiency/channels ratio (Quench Correction) curve for this counting system. Mean c.p.m.'s were thus converted to mean disintegrations per minute (d.p.m.) and the results expressed as d.p.m./ μ g DNA.

TABLE 1. The effects of steroid formulations on local (neck) and systemic (back) epidermal DNA synthesis 6 h after application

Steroid formulation*	DNA specific activity (d.p.m./ μ g DNA)					
	Steroid				Control	
	Neck Mean $\pm$ s.e.†	P	Back Mean $\pm$ s.e.†	P	Neck Mean $\pm$ s.e.†	Back Mean $\pm$ s.e.†
Efcortelan	94 $\pm$ 16	N.S.‡	136 $\pm$ 18	N.S.‡	119 $\pm$ 11	154 $\pm$ 17
Betnovate	79 $\pm$ 11	<0.01	115 $\pm$ 13	<0.05		
Molivate	80 $\pm$ 6	<0.01	152 $\pm$ 5	N.S.	124 $\pm$ 13	138 $\pm$ 16
Dermovate	53 $\pm$ 5	<0.001	99 $\pm$ 5	<0.05		
Synandone	61 $\pm$ 5	<0.01	103 $\pm$ 12	N.S.	106 $\pm$ 11	96 $\pm$ 11
Synalar	71 $\pm$ 4	<0.01	99 $\pm$ 14	N.S.	124 $\pm$ 14	100 $\pm$ 11
Florone	76 $\pm$ 12	<0.01	85 $\pm$ 16	N.S.	133 $\pm$ 9	122 $\pm$ 19

* Cream formulations except Synandone which is an ointment.

† n = 6.

‡ N.S. (not significant), $P > 0.05$.

TABLE 2. The effects of steroid formulations on local (neck) and systemic (back) epidermal DNA synthesis 24 h after application

Steroid formulation*	DNA specific activity (d.p.m./ μ g DNA)					
	Steroid				Control	
	Neck Mean $\pm$ s.e.†	P	Back Mean $\pm$ s.e.†	P	Neck Mean $\pm$ s.e.†	Back Mean $\pm$ s.e.†
Efcortelan	94 $\pm$ 13	<0.05	107 $\pm$ 25	N.S.‡	241 $\pm$ 57	101 $\pm$ 12
Betnovate	8 $\pm$ 2	<0.001	25 $\pm$ 7	<0.01	289 $\pm$ 51	149 $\pm$ 36
Molivate	31 $\pm$ 3	<0.001	129 $\pm$ 15	N.S.	227 $\pm$ 25	163 $\pm$ 18
Dermovate	26 $\pm$ 2	<0.001	37 $\pm$ 3	<0.001		
Synandone	5 $\pm$ 2	<0.001	86 $\pm$ 12	<0.05	206 $\pm$ 18	165 $\pm$ 27
Synalar	11 $\pm$ 2	<0.001	53 $\pm$ 7	<0.01		
Temetex	15 $\pm$ 6	<0.001	14 $\pm$ 5	<0.001	342 $\pm$ 85	90 $\pm$ 11
Florone	28 $\pm$ 3	<0.001	29 $\pm$ 3	<0.001	294 $\pm$ 29	129 $\pm$ 13

* Cream formulations except Synandone which is an ointment.

† n = 6.

‡ N.S. (not significant), $P > 0.05$.

RESULTS

The activities of the various formulations tested, at 6 and 24 h following initial application, are given in Tables 1 and 2.

Table 1 indicates that at 6 h after application all the formulations tested except Efcortelan had a significant local effect on the synthesis of DNA, the degree of inhibition ranging from 16 to 57%, and that Dermovate cream had a greater ($P < 0.05$) local effect than the other formulations. At this time all the formulations except Molivate and Efcortelan creams exhibited a systemic effect but this was only significant ($P < 0.05$) for Betnovate and Dermovate creams.

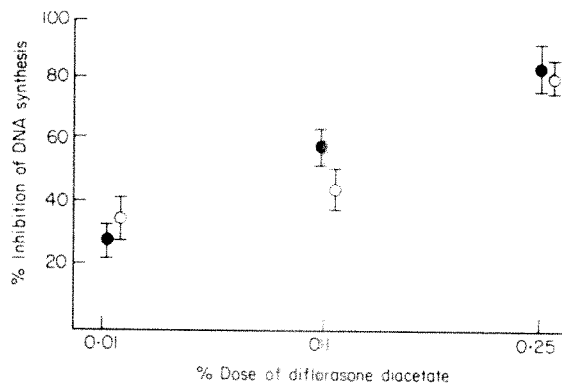


FIGURE 1. The effects of different concentrations of diflorasone diacetate, in alcoholic solution, on DNA synthesis. Results expressed as percent inhibition with respect to control mean. (●) Neck; (○) back, vertical bars, $\pm$ s.e.

At 24 h it was not possible to demonstrate any significant difference in local activity between Dermovate, Temetex, Synalar, Florone and Molivate creams and Synandone ointment. Efcortelan cream had a slight, significant effect on DNA synthesis at this time point.

At 24 h Dermovate, Temetex, Florone, Betnovate and Synalar creams and Synandone ointment all had a highly significant systemic effect. Molivate cream had no significant systemic effect despite a very highly significant local effect.

Fig. 1 illustrates the effect of 3 different concentrations of diflorasone diacetate, in alcoholic solution, on DNA synthesis. 0.25% had significantly greater local and systemic effects than 0.1%. Diflorasone diacetate 0.01% had a slight, but not statistically significant ($P > 0.05$) effect on DNA synthesis.

In these studies, by 24 h, Cream E45 had effected a significant increase in DNA synthesis in treated neck areas, with no effect in the untreated back areas (see Tables 1 and 2). Table 3 shows the

TABLE 3. The effect of different 'control' treatments on epidermal DNA synthesis 24 h after treatment

Treatment	DNA specific activity (d.p.m./ μ g DNA)			
	Neck Mean $\pm$ s.e. *	P	Back Mean $\pm$ s.e. *	P
Untreated	96 $\pm$ 10		123 $\pm$ 8	
(a) Rubbing alone	98 $\pm$ 13	N.S.†	124 $\pm$ 15	N.S.†
(b) Molivate base	121 $\pm$ 15	N.S.	124 $\pm$ 18	N.S.
(c) Cream E45	194 $\pm$ 14	< 0.001	128 $\pm$ 11	N.S.

* n = 6.

† N.S. (not significant), $P > 0.05$.

results of an experiment devised to show the effects in the neck skin, compared to an untreated control, of (a) rubbing with the seeker alone (b) application of the vehicle used in the formulation of Molivate cream and (c) application of Cream E45.

It is confirmed that by 24 h after application, Cream E45 promoted an increase in DNA synthesis when compared with untreated skin. Rubbing alone with a seeker or the application of Molivate base had no significant effect.

DISCUSSION

The vasoconstrictor bioassay for topical glucocorticosteroids (McKenzie & Stoughton, 1962), although simple and reliable, has certain limitations; (a) the potential for systemic toxicity is not predictable, (b) the test applies to steroids only and (c) the vasoconstrictor action *per se* may not be related to the therapeutic effect desired by the prescribing clinician. Thus a more specific assay was sought. The animal chosen for an assay of the DNA synthesis inhibiting effects of steroids and anti-metabolites was the hairless mouse. This animal was selected because it possesses fewer viable hair follicles than normal animals and these therefore probably play a smaller role in percutaneous absorption. The tissue damaging processes of depilation or clipping and shaving are obviated and the animal is easily bred and handled. In comparative studies using hairless mouse skin and human skin, similar transcutaneous diffusion rates were obtained for several drugs (Stoughton, 1975).

The system described was a development of that employed previously by du Vivier *et al.* (1976) and was designed to screen both steroids and their formulations. In this study, because comparative efficacy of *formulations* was being assayed, control animals were treated with a simple emollient cream rather than respective formulation bases since the bases themselves may possess inherent activity. The results thus obtained reflect the activity of the formulation *in toto* rather than that of the steroid alone and can thus be meaningfully related to the clinical situation.

At 6 h Dermovate cream had a significantly greater effect than the other steroids tested. This, however, would be expected in light of clinical experience and data from vasoconstrictor bioassay studies. Corticosteroid formulations may be assigned to one of three main classes, according to activity—highly potent, moderately potent and weak. Dermovate cream would be classed as highly potent, Betnovate, Synalar, Florone and Molivate creams as moderately potent, and Efcortelan cream as a weak formulation. It is of interest that the ointment formulation of Synandone (0.01% flucinolone acetone) exhibited comparable activity to the cream formulation of Synalar (0.025% flucinolone acetone). This shows the marked influence of formulation on the bioavailability of the steroid, the difference in concentration being compensated for by the occlusive properties and/or drug-partitioning characteristics of the ointment formulation.

At 24 h all the steroid formulations, except Efcortelan, produced greater than 80% inhibition of DNA synthesis when compared to the control formulation. This indicates that the steroid formulations tested are too powerful, in this animal model, for differences between them to be shown at the 24 h time point.

The best interpretation of results, therefore, is to take measurements 6 h after application of the drug formulation for assessment of local activity. Dilution of simple alcoholic solutions could be used for screening steroids *per se* for activity, but dilution of marketed formulations would be inadvisable since it may alter their intrinsic drug-releasing characteristics. For a more informative comparison between formulations, and to allow maximum use of statistical interpretations a Latin Square format could be used, with more animals *per group*.

The model is undoubtedly useful in predicting systemic effects of a steroid. At 6 h both Betnovate and Dermovate creams already show a systemic effect. This may reflect a higher transcutaneous flux of steroid from these formulations. At 24 h Dermovate cream has effected very highly significant inhibition of DNA synthesis throughout the epidermis of the animal, as has Temetex cream. Adverse reports on the new formulation, Temetex cream, have not yet appeared but systemic side effects from topically applied Dermovate have been reported on several occasions (Feiweil & Kelly, 1974; Staughton & August, 1975; Ortega, Burdick & Segre, 1975; Boxley, Dawber & Summerly, 1975; Carruthers, August & Staughton, 1975) whereas for Betnovate and Synalar, although known to have such potential, there are only occasional reports (Keipert & Kelly, 1971; Kelly *et al.*, 1972).

Molivate is unusual in that, although being classed as a moderately potent steroid, it has no systemic effect in our model system. This is surprising since it is usual experience that the greater the vasoconstrictor activity of a compound the greater the potential for systemic activity and Molivate is almost as potent as Betnovate in the vasoconstrictor bioassay. However, this finding in our model confirms those of Munro & Wilson (1975). In studies of hypothalamic-pituitary-adrenal axis function under conditions predisposing to maximum absorption of the drug, namely extensive skin disease and polythene occlusion, they concluded that a minimal effect with Molivate was observed. This was in marked contrast to Betnovate and Dermovate. Other animal studies similarly confirm this finding. Child *et al.* (1968) described a technique for determining systemic activity by measuring the effect of subcutaneously administered steroids on the weight of the thymus gland in mice and rats. In such a test system Betnovate and Synalar have similar thymolytic activity. The effect of Dermovate is 3–4 times as great as that of Betnovate. Molivate appears to have no thymolytic activity thereby supporting our findings.

Temetex is a new steroid formulation. It is effective in the treatment of psoriasis (Wilkinson & de la Ferla, 1975) and produces a more rapid clinical response than Betnovate (Leu, 1976). On the basis of our studies it would appear to be a moderately potent steroid. Although to date no systemic effects of Temetex have been reported, the risk of systemic toxicity, if injudiciously prescribed, is high.

It is considered that an effect at a site distal to that of application of the drug formulation reflects systemic activity. This consideration is supported by the fact that there is no (macroscopically) visible spread of formulation from the treated neck area to the skin of the back, either as indicated by glistening of the skin or transfer of dyes incorporated in the test formulations. Further, the animals were immobilized for a period after application so that they could not spread the formulation by rubbing or ingesting it. Weight loss appears to occur in animals treated with topically applied steroids, when inhibition of DNA synthesis is found in an untreated site, but not in control animals (unpublished observations). Weight loss did not occur in Molivate (cream) treated animals. Finally, Table 3 shows that the application of a formulation such as Cream E45 causes an increase only in local DNA synthesis. If spreading were to occur an increase would also be observed in the back, but this is not seen. This adverse effect of Cream E45 indicates how the model might also be used to determine such an effect of a vehicle.

Florone, a new steroid currently undergoing evaluation, is evidently a potent inhibitor of epidermal DNA synthesis. The findings using different concentrations of this steroid support those of Marks, Pongsehirum & Saylan (1973) that the effect of steroids on cell replication is not an all or none effect. As the concentration is reduced from 0.25% to 0.01% a significant reduction in both local and systemic activity occurs, thereby demonstrating a dose related effect in this animal model.

In conclusion, the hairless mouse model described may be used as a simple system for determining the degree of local effect of a steroid or steroid formulation on epidermal DNA synthesis and simultaneously to predict its potential for systemic activity.

REFERENCES

- BOXLEY, J.D., DAWBER, R.P.R. & SUMMERLY, R. (1975) Generalised pustular psoriasis on withdrawal of clobetasol propionate ointment. *British Medical Journal*, **ii**, 255.
- CARRUTHERS, J.A., AUGUST, P.J. & STAUGHTON, R.C.D. (1975) Observations on the systemic effect of topical clobetasol propionate (Dermovate). *British Medical Journal*, **iv**, 203.
- CHILD, K.J., ENGLISH, A.F., GILBERT, H.G., HEWITT, A. & WOOLLETT, E.A. (1968) Vasoconstrictor and systemic activities of topical steroids. *Archives of Dermatology*, **97**, 407.
- DU VIVIER, A., BIBLE, R., MIKURIYE, R. & STAUGHTON, R.B. (1976) An animal model for screening drugs for anti-psoriatic properties using hydroxyapatite to rapidly isolate DNA from the epidermis. *British Journal of Dermatology*, **94**, 1.
- FEIWEL, M. & KELLY, W.J. (1974) Adrenal unresponsiveness associated with clobetasol propionate. *Lancet*, **ii**, 112.
- FELSENFIELD, G. & HIRSCHMAN, S.Z. (1965) A neighbour-interaction of the hyperchromism and spectra of DNA. *Journal of Molecular Biology*, **13**, 407.
- KEIPERT, J.A. & KELLY, R. (1971) Temporary Cushing's syndrome from percutaneous absorption of beta-methasone-17-valerate. *Medical Journal of Australia*, **1**, 542.
- KELLY, A., NELSON, K., GOODWIN, M. & MCCLUGGAGE, J. (1972) Iatrogenic Cushing's syndrome. *British Medical Journal*, **iv**, 114.
- LEU, F. (1976) Évaluation dermatologique comparative de deux préparations de corticostéroïdes locaux. *Revue Médicale de la Suisse Romande*, **96**, 323.
- MARKS, R., PONGSEHIRUM, D. & SAYLAN, T. (1973) A method for the assay of topical corticosteroids. *British Journal of Dermatology*, **88**, 69.
- MCKENZIE, A.W. & STAUGHTON, R.B. (1962) Methods for comparing percutaneous absorption of steroids. *Archives of Dermatology*, **108**, 43.
- MEINKE, W., GOLDSTEIN, D.A. & HALL, M.R. (1974) Rapid isolation of mouse DNA from cells in tissue culture. *Analytical Biochemistry*, **58**, 82.
- MUNRO, D.D. & WILSON, L. (1975) Clobetasone butyrate, a new topical corticosteroid: clinical activity and effects on pituitary-adrenal axis function and model of epidermal atrophy. *British Medical Journal*, **iii**, 625.
- ORTEGA, E., BURDICK, K.H. & SEGRE, E.J. (1975) Adrenal suppression by clobetasol propionate. *Lancet*, **i**, 1200.
- STAUGHTON, R.C.D. & AUGUST, P.J. (1975) Cushing's syndrome and pituitary-adrenal suppression due to clobetasol propionate. *British Medical Journal*, **ii**, 419.
- STAUGHTON, R.B. (1975) Animal models for *in vitro* percutaneous absorption. In: *Animal Models in Dermatology* (Ed. by Howard Maibach), p. 121. Churchill Livingstone, Edinburgh.
- WILKINSON, F.F. & DE LA FERLA, G.P. (1975) Empleo de la difluorcortolona en dermatología. Paper read at 1st. Latin American Congress for Dermatological Therapy, Buenos Aires.

Case Reports

Acute adult-onset Darier-like dermatosis

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SUMMARY

We describe 2 patients with a dermatosis like Darier's disease and resembling it histologically. However, the eruption was characterized by adult-onset and rapid spread.

Darier's disease (dyskeratosis follicularis) is a rare hereditary dermatosis, usually presenting before adolescence (Ebling & Rook, 1972; Svendsen & Albrechtsen, 1959). It has been considered a distinct entity. In recent years it has, however, become apparent that adult-onset conditions exist with varying clinical and histological similarities (Fishman, 1975; Grover, 1970; Heaphy, Tucker & Winkelmann, 1976). We shall here describe observations in 2 such patients.

CASE REPORTS

Case 1

An 82-year-old man. There is no family history of any skin disease. The past medical history was normal, except for a salicylate precipitated acute urticaria at the age of 50. He was never treated with arsenic. In April 1973, an adenocarcinoma of the stomach was excised. It had penetrated to the serosal surface but no abdominal metastases could be found. A pulmonary X-ray was normal. Some weeks after the operation, he gradually developed brownish, slightly red, hyperkeratotic, non-pruritic papules on the upper part of the trunk and the lower parts of the neck, the upper arms and the back of the hands and fingers (Fig. 1). They did not have the clinical appearance of seborrhoeic keratoses. The groins and axillae were spared. There were no palmoplantar or nail abnormalities. In September 1973, he was examined at the Department of Dermatology, Sahlgren's Hospital. Abnormal findings were essentially confined to these skin lesions and a 2 × 2 cm superficial basal cell carcinoma on the right scapula (diagnosis verified by skin biopsy); it was removed by curettage and electro-desiccation. He was not taking any drugs. Normal laboratory investigations included complete blood cell count, ESR, serum bilirubin, alkaline phosphatase, thymol, SGOT, SGPT, serum creatinine, Wasserman test, serum concentrations of vitamin A and beta-carotene. Results of urinalysis were also normal. No systemic or topical treatment was given. At the next examination, in February 1974,

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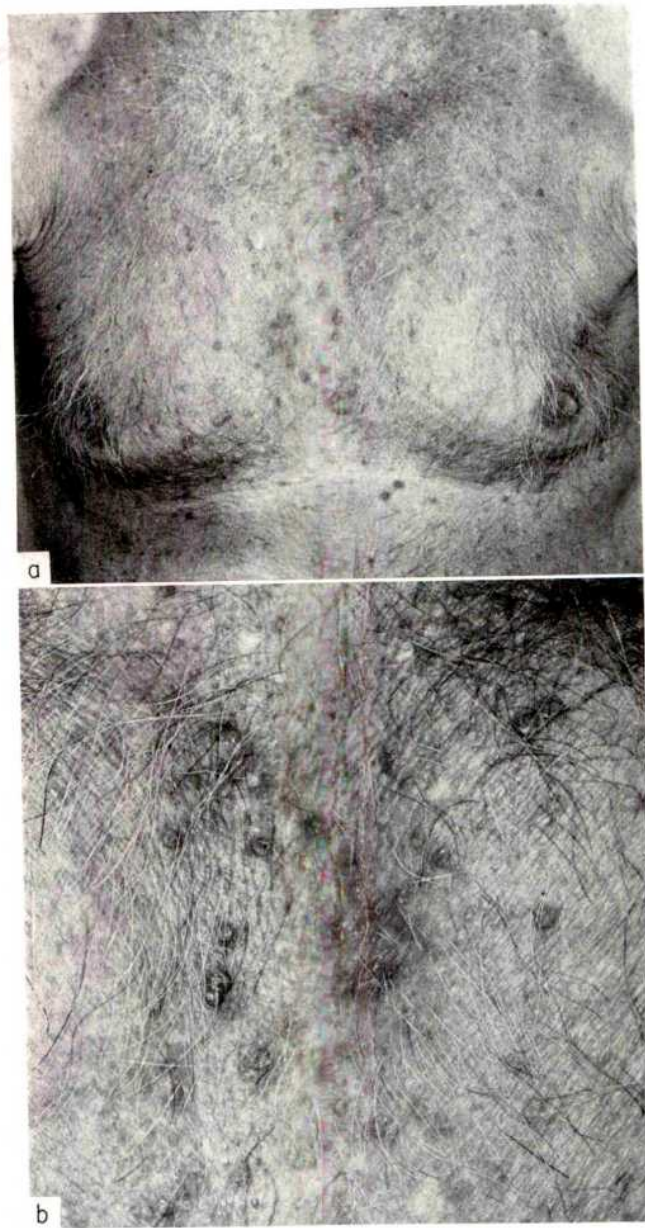


FIGURE 1. (a and b) Skin lesions of Case I.

the number and appearance of the skin lesions were unchanged. Later he became icteric. The skin lesions persisted to his death, in October 1974, of liver metastases from the gastric carcinoma.

Histopathology. Three skin biopsy specimens were taken from the trunk on separate occasions. The specimens showed a hyperkeratotic and papillomatous epidermis with the formation of keratotic

plugs. Suprabasal lacunae lined with a single layer of basal cells were seen under the keratotic plugs. There were dyskeratotic cells with formation of grains and corps ronds, and perifollicular foci of acantholysis. In the upper corium there was a perivascular infiltrate of lymphocytes and histiocytes.

Case 2

A 50-year-old man. His family history is normal. He has for many years had attacks of low back pain, diagnosed as lumbago. He admits to a high, regular ethanol consumption. In November 1976, he developed itching skin lesions on the back. When he was examined 4 weeks after the onset he had reddish-brown, partly confluent papules densely distributed on the trunk but no intertriginous, mucosal, palmoplantar or nail lesions. He was not taking any drugs. Normal laboratory studies included complete blood cell count, serum creatinine, Wasserman test and antinuclear factor. Pulmonary X-ray, normal.

Towards the end of December 1976, he suddenly developed a right hemiparesis and dysphasia. He has since been treated at the Department of Neurology. Angiography reveals total occlusion of the left internal carotid artery.

Histopathology. Two skin biopsies from the trunk both showed epidermal hyperkeratosis, papillomatosis and hyperkeratotic plugs. There were foci of suprabasal acantholytic lacunae. Dyskeratotic cells in the form of corp ronds and grains were seen.

Immunofluorescence studies. Skin biopsy specimens of abnormal and uninvolved skin were investigated by direct immunofluorescence for deposits of immunoglobulins and complement. IgG, IgA, IgM and C3 could not be demonstrated.

DISCUSSION

The clinical features of the profuse eruption on the trunk of Case 2 are characteristic of Darier's disease. In Case 1, the moderately dense eruption was compatible with an early case of Darier's disease but a Leser-Trelat syndrome was also considered initially (Dantzig, 1973). The histological findings and the absence of body-fold lesions distinguish Case 1 from the syndrome of malignant acanthosis nigricans with verrucous lesions (White, 1976). The findings of the multiple histological examinations in both our patients are diagnostic of Darier's disease (Pinkus & Mehregan, 1969). The cases differ, however, in some important aspects from patients with Darier's disease: (1) Onset in advanced age is rare although it does not exclude this diagnosis (Ebling & Rook, 1972; Fishman, 1975; Witten, 1963). (2) Our cases exhibited rapid spread of lesions, unlike the slow spread over years usually seen in Darier's disease (Svendsen & Albrechtsen, 1959). It should be noted that they had not been exposed to sunlight shortly before or after the onset, a known provoking factor (Svendsen & Albrechtsen, 1959). (3) The 2 patients had no affected relatives. Darier's disease is an autosomal dominantly inherited disease and there is still no genetic marker known for it, e.g. in the HLA system.

Recently some conditions have been reported which histologically may simulate classical Darier's disease but can be differentiated clinically (Chalet *et al.*, 1977; Fishman, 1975; Grover, 1970; Heaphy *et al.*, 1976). The dense, partly confluent eruption in Case 2 differs from the picture seen in transient acantholytic dermatosis (TAD).

As for Case 1, absence of itchiness is uncommon in TAD (Chalet *et al.*, 1977). Persistence of the lesions in TAD does occasionally occur, but most patients have a brief course (Chalet *et al.*, 1977; Simon *et al.*, 1976). Pronounced dyskeratosis with corps ronds and grains, as found in our cases, is not seen in benign papular acantholytic dermatosis (Heaphy *et al.*, 1976).

Summing up, it cannot be excluded that our patients are atypical phenotypes of Darier's disease. The clinical and histological diversity of recently published case reports suggests, however, that they may represent a cutaneous reaction pattern which may be triggered by different pathogenic events (cf. psoriasiform, lichenoid, urticarial reactions (Ackermann, 1972; Heaphy *et al.*, 1976). They are likely to be more common than the literature suggests.

REFERENCES

- ACKERMANN, A.B. (1972) Focal acantholytic dyskeratosis. *Archives of Dermatology*, **106**, 702.
- CHALET, M., GROVER, R. & ACKERMANN, A.B. (1977) Transient acantholytic dermatosis. A re-evaluation. *Archives of Dermatology*, **113**, 431.
- DANTZIG, P.I. (1973) Sign of Leser-Trelat. *Archives of Dermatology*, **108**, 700.
- EBLING, F.J. & ROOK, A. (1972) Disorders of keratinization. In: *Textbook of Dermatology* (ed. by A.Rook, D.S. Wilkinson and F.J.Ebling) 2nd edn, p. 1169. Blackwell Scientific Publications, Oxford.
- FISHMAN, H.C. (1975) Acute, eruptive Darier disease (keratosis follicularis). Occurrence in an adult. *Archives of Dermatology*, **111**, 221.
- GROVER, R.W. (1970) Transient acantholytic dermatosis. *Archives of Dermatology*, **101**, 426.
- HEAPHY, M.R., TUCKER, S.B. & WINKELMANN, R.K. (1976) Benign papular acantholytic dermatosis. *Archives of Dermatology*, **112**, 814.
- PINKUS, H. & MEHREGAN, A.H. (1969) *A Guide to Dermatohistopathology*. Butterworths, London.
- SIMON, R.S., BLOOM, D. & ACKERMAN, A.B. (1976) Persistent acantholytic dermatosis. A variant of transient acantholytic dermatosis (Grover disease). *Archives of Dermatology*, **112**, 1429.
- SVENDSEN, I.B. & ALBRECHTSEN, B. (1959) The prevalence of dyskeratosis follicularis (Darier's disease) in Denmark. *Acta Dermato-venereologica*, **39**, 256.
- WHITE, H. (1976) Acanthosis nigricans and wart-like lesions associated with metastatic carcinoma of the stomach. *Cutis*, **17**, 931.
- WITTEN, V.H. & GELFARB, M. (1963) Keratosis follicularis of sudden onset. *Archives of Dermatology*, **87**, 732.

Multicentric reticulohistiocytosis and malignant disease

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SUMMARY

Multicentric reticulohistiocytosis developed in a 45-year-old woman, who had a 3 year history of untreated breast carcinoma. A review of the literature revealed that malignant disease has been associated with 27% of reported cases. It is considered that, like adult dermatomyositis, the onset of multicentric reticulohistiocytosis should stimulate detailed investigation for an underlying neoplasm.

Multicentric reticulohistiocytosis (MR) is a rare disorder, with a predilection for middle aged women and characterized by polyarthritis and papulo-nodular skin lesions. Histologically, histiocytic multinucleate giant cells are the structural hallmark in skin, synovia and other involved tissues. The disease has been reviewed by Barrow & Holubar (1969) and its tendency to multisystem involvement re-emphasized by Fast (1976). We report a typical case which highlights the association with malignant disease.

CASE REPORT

N.D., a 46-year-old woman, presented in October 1973 with a Stage III scirrhous carcinoma of her right breast, for which she refused treatment. In March 1976 she was referred with a 1 month history of a progressive papulo-nodular eruption, which had appeared first on her face and neck and spread caudally. She now consented to bilateral oophorectomy and needle biopsy of her breast, which confirmed the clinical diagnosis. Following surgery, she developed acute polyarthralgia and began to lose weight.

Physical examination revealed a thin woman, with obvious Stage III breast carcinoma. There were numerous dusky, erythematous cutaneous papules and nodules, 0.5 mm–4 cm in diameter, affecting the face, neck, ears, elbows, hands (Fig. 1), buttocks, knees and oral mucosa. Signs of arthritis were apparent in the shoulders, elbows, wrists and hands, knees, ankles and feet.

Since the onset of the disease, some skin lesions have spontaneously resolved, but the arthropathy has been progressive and particularly mutilating to the wrists and hands, resulting in 'concertina' deformity of several fingers. Systemic corticosteroid therapy was started early, but its effect has been difficult to assess and certainly not dramatic.

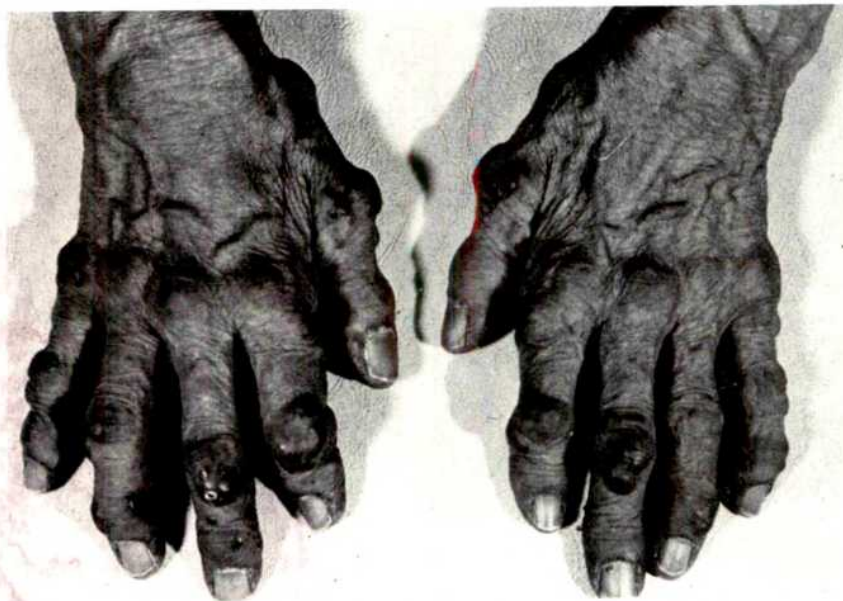


FIGURE 1. Multiple nodules and papules on the backs of both hands.

INVESTIGATIONS

Normal blood parameters included white cell count, ESR., urea and electrolytes, liver function tests, glucose, calcium, uric acid, cholesterol and triglycerides, proteins and electrophoresis, immunoglobulins, C₃ and C₄, lymphocyte receptor studies and thyroid function tests. Auto-immune profile, including rheumatoid factor, was negative. Tests of renal function and intestinal absorption, including jejunal biopsy were normal. Chest X-ray, barium meal, skeletal survey for metastases and a bone scan revealed nothing of significance. A Mantoux test (1:1000) and Kveim test were negative and the E.C.G. normal. Haemoglobin, serum iron, and iron binding capacity were all persistently low and bone marrow examination revealed depleted iron stores, but no evidence of infiltration by either carcinoma, or reticulohistiocytosis.

X-rays of joints demonstrated numerous periarticular erosions in the wrists and hands (Fig. 2), with particularly marked derangement of the distal interphalangeal joints. Prominent erosive changes were also apparent in the forefeet and similar but less striking changes in shoulders, elbows, knees and ankles.

Several nodules were removed for histological examination and all showed a prominent dermal infiltrate composed mainly of histiocytes and multinucleate giant cells, with weakly PAS positive, diastase resistant cytoplasm (Fig. 3).

Tissue lipid studies of involved skin, using thin layer chromatography, demonstrated a normal distribution of phospholipids, neutral lipids, gangliosides and glycolipids, with no excess of any one fraction.

DISCUSSION

Of 41 cases of MR in the literature, 11 (27%) have now been associated with malignant disease. With one exception, a patient with axillary sarcoma (Albert *et al.*, 1960), the neoplasms have all been



FIGURE 2. X-ray of hands, showing numerous peri-articular erosions and soft tissue swellings. Several distal interphalangeal joints are completely deranged.



FIGURE 3. High power view of H & E section, showing the prominent dermal histiocytic infiltrate, with numerous multinucleate giant cells.

carcinomata. Apart from the breast, primary tumours have affected colon (Goltz & Laymon, 1954), bronchus (Warin *et al.*, 1957), stomach (Albert *et al.*, 1960), ovary (Hall-Smith, 1976) and cervix (Holubar & Mach, 1966; Hanauer, 1972). One patient died of carcinomatosis, the primary remaining undetected (Labow & Shapiro, 1965).

There is only one previous formal report of the disease associated with breast carcinoma (Granelli, Bignami & Nazarro, 1963), but a second case was noted by Labow & Shapiro (1965), having been reported to them by colleagues.

Like adult dermatomyositis, a well recognized manifestation of malignancy, it is clear that the onset of MR may antedate detection of the neoplasm by a considerable period. Thus, one of the four patients reviewed by Warin *et al.* (1957) developed MR in 1950 and not until post mortem examination 4 years later was an underlying bronchial carcinoma detected. The patient reported by Hall-Smith (1976) was found to have a medullary ovarian carcinoma 7 months after presenting with typical lesions of MR.

The presence of malignant disease in patients with MR has largely been dismissed as coincidence, but we feel the disease should, like dermatomyositis, now be considered a rare manifestation of malignancy and since it may precede detection of the neoplasm by months or years, its onset should stimulate detailed investigation.

ACKNOWLEDGMENTS

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REFERENCES

- ALBERT, J., BRUCE, W., ALLEN, A.C. & BLANK, H. (1960) Lipoid dermatoarthritis. Reticulohistiocytoma of the skin and joints. *American Journal of Medicine*, **28**, 661.
- BARROW, M.V. & HOLUBAR, K. (1969) Multicentric reticulohistiocytosis, a review of 33 patients. *Medicine*, **48**, 287.
- FAST, A. (1976) Cardiopulmonary complications of multicentric reticulohistiocytosis. *Archives of Dermatology*, **112**, 1139.
- GOLTZ, R.W. & LAYMON, C.W. (1954) Multicentric reticulohistiocytosis of the skin. *Archives of Dermatology and Syphilology*, **69**, 717.
- GRANELLI, U., BIGNAMI, A. & NAZZARO, P. (1963) Reticuloistocitosi gigantocellulare (dermato-artrite lipoidea). *Giornale Italiano Di Dermatologia*, **104**, 285.
- HALL-SMITH, P. (1976) Multicentric reticulohistiocytosis with ovarian carcinoma. *Proceedings of the Royal Society of Medicine*, **69**, 380.
- HANAUER, L.B. (1972) Reticulohistiocytosis. Remission after cyclo-phosphamide therapy. *Arthritis and Rheumatism*, **15**, 636.
- HOLUBAR, K. & MACH, K. (1966) Giant cell histiocytosis. A contribution to the clinical and histological aspects. *Hautarzt*, **17**, 440.
- LABOW, T.A. & SHAPIRO, L. (1965) Giant cell reticulohistiocytoma associated with cancer. *New York Journal of Medicine*, **65**, 2941.
- WARIN, R.P., EVANS, C.D., HEWITT, M., TAYLOR, A.L., PRICE, C.H.G., & MIDDLEMISS, J.H. (1957) Reticulohistiocytosis (lipoid dermato-arthritis). *British Medical Journal*, **i**, 1387.

Photosensitivity after treatment of porphyria cutanea tarda with low dose chloroquine

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SUMMARY

Treatment of a patient with porphyria cutanea tarda with low dose chloroquine improved the clinical and biochemical abnormalities in association with a reduction in photosensitivity as shown by changes in the action spectrum.

Low dose chloroquine in the treatment of porphyria cutanea tarda (PCT) corrects the clinical and biochemical abnormalities associated with this disorder (Saltzer, Redeker & Wilson, 1968; Taljaard *et al.*, 1972; Kordac & Semradova, 1974). Although clinical photosensitivity has been noted to improve with this treatment, objective measurements of photosensitivity have not been undertaken. Recently we performed these on a patient.

A 70-year-old retired male factory worker with a high alcoholic intake presented with the clinical features of PCT which was confirmed by biochemical investigations (Table 1) using the method of Rimington (1971). Serum iron and AST (SGOT) were normal, however the γ -glutamyl transpeptidase was slightly elevated at 84 units (normal < 70 units). An action spectrum was carried out with the irradiation monochromator and employing the technique of Mackenzie & Frain-Bell (1973). This demonstrated an increased sensitivity to irradiation in the ultraviolet and visible spectra, with

TABLE 1. Porphyrin patient

	Urine	
	Uroporphyrin ($\mu\text{g}/24\text{ h}$)	Coproporphyrin ($\mu\text{g}/24\text{ h}$)
Normal	0-41	0-283
Patient	583	198

markedly increased sensitivity in the 365-430 nm range (Fig. 1). Oral chloroquine was given in a dosage of 77.5 mg of chloroquine base twice weekly. Regular porphyrin estimations on urine and

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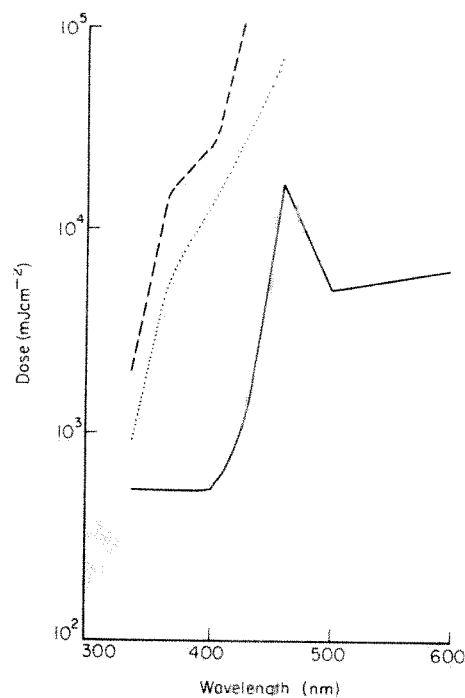


FIGURE 1. Illustrates the progressive reduction in photosensitivity from the pre-treatment period (—) to the period 3 months after starting chloroquine treatment (.....) to the period 4 months after ceasing treatment (---).

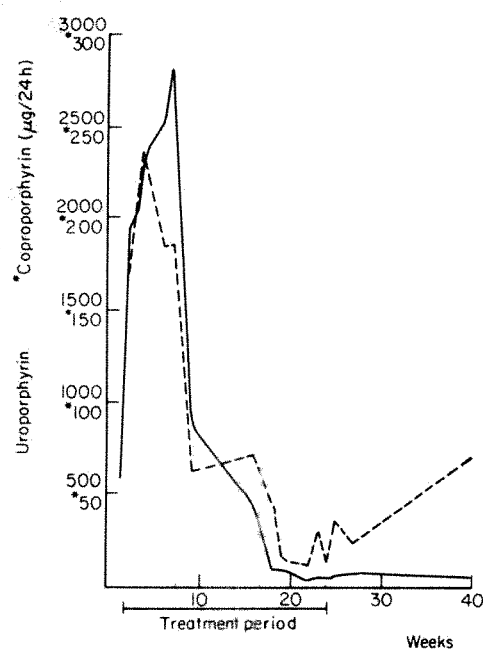


FIGURE 2. There are marked reductions in urinary uroporphyrin (—) and coproporphyrin (---) levels as a result of chloroquine treatment.

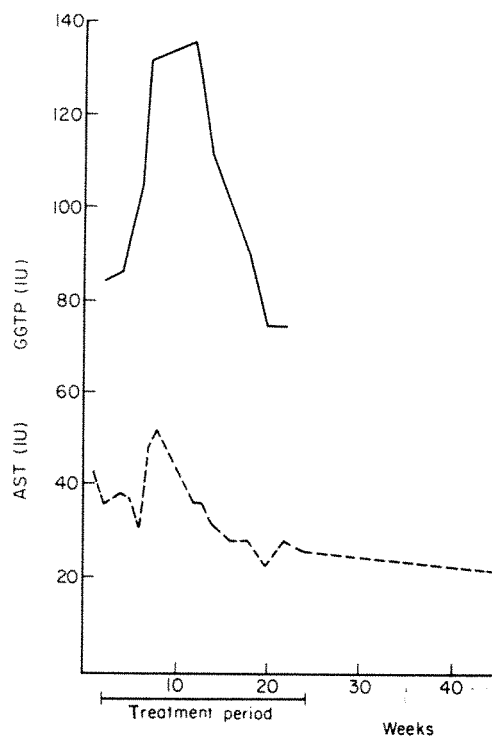


FIGURE 3. There are variations in serum AST (---) and γ -glutamyl transpeptidase (—) in association with and after chloroquine administration.

stool samples and liver function tests, including transaminases and γ -glutamyl transpeptidase, were carried out during and after the treatment (Figs 2 and 3).

Within 1 month the patient was feeling better and had observed a reduction in skin fragility with cessation of blister formation. He progressively improved, the treatment was discontinued after 5 months and he has remained free of symptoms for a further 4 months.

Action spectrum studies showed a progressive reduction in sensitivity especially in the 400 nm region throughout the treatment period, and this has been maintained in the post-treatment period (Fig. 1). Three months after starting chloroquine treatment the threshold dose for erythema at 400 ± 30 nm had increased by a factor of 27 (560 mJ/cm^2 to $15,000 \text{ mJ/cm}^2$). At the completion of the treatment the threshold dose for erythema at 400 ± 30 nm had increased by a factor of 39 (560 mJ/cm^2 to $22,000 \text{ mJ/cm}^2$). Four months after stopping treatment, the threshold dose for erythema at 400 ± 30 nm had increased by a factor of 48 (560 mJ/cm^2 to $27,000 \text{ mJ/cm}^2$).

DISCUSSION

Treatment of PCT with chloroquine has not had a wide application because of the frequent incidence of severe side effects resulting from the release of large quantities of porphyrin from the liver (Felsner & Redeker, 1966). However, more recent reports have suggested that chloroquine in much smaller doses is acceptable without adverse side effects.

Irradiation monochromator studies of the skin of patients with PCT have disclosed an increased sensitivity at about 400 nm and sometimes at 500–600 nm (Magnus, 1974). Action spectrum studies of

patients treated with phlebotomy have shown as a result of this treatment a reduced skin response at 400 nm peak (Ramsay *et al.*, 1974).

Treatment with low dose chloroquine in the patient described in this report resulted in a remarkable reduction of the threshold erythematous responses within 3 months of starting the treatment. This reduction has persisted after the treatment had been stopped for 4 months and was associated with clinical and biochemical remission.

REFERENCES

- FELSHER, B.F. & REDEKER, A.G. (1966) Effect of chloroquine on hepatic uroporphyrin metabolism in patients with porphyria cutanea tarda. *Medicine*, **45**, 575.
- KORDAC, V. & SEMRADOVA, M. (1974) Treatment of porphyria cutanea tarda with chloroquine. *British Journal of Dermatology*, **90**, 95.
- MACKENZIE, L.A. & FRAIN-BELL, W. (1973) The construction and development of a grating monochromator and its application to the study of the reaction of the skin to light. *British Journal of Dermatology*, **89**, 251.
- MAGNUS, I.A. (1974) Porphyria as a model of abnormal skin sensitivity. Parkes Weber Lecture. In: *10th Symposium on Advanced Medicine, Proceedings of a Conference held at the Royal College of Physicians of London 18-22nd February 1974* (ed. by J.G.G. Ledingham), p. 333. Pitman Medical, London.
- RAMSAY, C.A., MAGNUS, I.A., TURNBULL, A. & BARKER, H. (1974) The treatment of porphyria cutanea tarda by venesection. *Quarterly Journal of Medicine*, **XLIII**, 1.
- RIMINGTON, C. (1971) Quantitative determinations of porphobilinogen and porphyrins in urine and porphyrins in faeces and erythrocytes. Broadsheet 70, *Association of Clinical Pathology*.
- SALTZER, E.I., REDEKER, A.G. & WILSON, J.W. (1968) Porphyria cutanea tarda. Remission following chloroquine administration without adverse effects. *Archives of Dermatology*, **98**, 496.
- TALJAARD, J.J.F., SHANLEY, B.C., STEWART-WYNNE, E.G., DEPPE, W.M., & JOUBERT, S.M. (1972) Studies on low dose chloroquine therapy and the action of chloroquine in symptomatic porphyria. *British Journal of Dermatology*, **87**, 261.

Opportunistic infection of chronic skin ulcers with *Pseudomonas putrefaciens*

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SUMMARY

Heavy growth of *Pseudomonas putrefaciens* was isolated repeatedly in mixed culture with other Gram-negative rods from chronic ulcers on the extremities of an elderly patient with burnt-out leprosy. Treatment was with systemic cotrimoxazole, topical framycetin and general supportive therapy, and the ulcers gradually healed over a period of 4 weeks.

Organisms of the genus *Pseudomonas* are mostly saprophytes with a wide distribution in nature. Opportunistic infection with these bacteria may occur especially in debilitated patients and areas of decreased blood supply (Debois *et al.*, 1975; Gilardi, 1976). *Pseudomonas putrefaciens* is an example of this bacterial group. The pathogenic nature of this organism has been questioned (Von Graevenitz & Simon, 1970; Gilardi, 1972; Debois *et al.*, 1975; Gilardi, 1976). This report describes a patient with chronic ulcers on the extremities which yielded repeated heavy growth of *P. putrefaciens* in mixed culture with other Gram-negative rods.

CASE REPORT

A 60-year-old African woman with a previous history of successfully treated leprosy was admitted to King Edward VIII Hospital in congestive cardiac failure. The patient was in poor general condition and physical examination showed marked evidence of leprosy bone resorption in both feet. Signs of cardiac failure included basal crepitations in both lungs, ankle oedema, orthopnoea, congested neck veins, and hepatomegaly. Two skin ulcers were observed, one on the left wrist (c 1 cm diameter) the other below the left knee (c 2 cm diameter). Both ulcers were relatively deep (c 2–3 mm), had sharply defined edges, and were covered with a purulent granulating exudate. Marked trophic changes were seen in both legs. Interrogation was complicated by the patient's confused mental state, but revealed that both ulcers had been present for several months prior to admission. Initial laboratory investigations revealed an ESR of 36 mm/h and a white cell count of 8000/mm³; electrolytes and urea were within normal limits.

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Pus swabs taken on admission revealed profuse growth of a *Pseudomonas* species (later identified as *P. putrefaciens*) from both ulcers—other organisms isolated (all in much smaller quantities) included *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and a *Moraxella* species. Subsequent swabs taken from these sites on 5 different occasions during the first week all yielded heavy growth of the *Pseudomonad* with scanty growth of various combinations of *Enterobacteria* listed above. Therapy for heart failure, together with a well-supplemented diet, improved the patient's general condition. Three days after admission treatment with cotrimoxazole (2 tablets twice daily for 10 days, followed by 1 tablet twice daily for a similar period) was instituted; ulcers were treated topically with tulle gras dressings containing framycetin. Five days after commencement of antimicrobial therapy isolation of Gram-negative rods from the lesions ceased and healing gradually began to take place; subsequent swabs from both ulcer sites yielded *Staphylococcus epidermidis* and diphtheroid organisms. The patient was discharged 4 weeks later in reasonably good physical condition.

Bacteriology

Organisms were isolated and identified as described previously (Debois *et al.*, 1975; Gilardi, 1976). The most salient feature of this organism was the heavy blackening of stabbed Kligler iron agar due to H_2S -production after overnight incubation. This characteristic led in some cases to a mistaken preliminary identification of the organism as *Citrobacter* or *Salmonella*. Antibiotic susceptibility testing by the disc method (Stokes, 1975) showed the organism to be sensitive to tetracycline (10 µg), streptomycin (10 µg), chloramphenicol (10 µg), gentamicin (10 µg), cotrimoxazole (25 µg), ampicillin (10 µg), carbenicillin (100 µg), erythromycin (5 µg) and resistant to penicillin G (2 units), methicillin (10 µg), sulphonamide (100 µg), cephalosporin (30 µg). All other organisms isolated were sensitive to cotrimoxazole, streptomycin and gentamicin, with differing susceptibilities to other antimicrobials, in disc diffusion tests.

DISCUSSION

Pseudomonas putrefaciens has a wide distribution in nature, its natural habitat being water and soil (Gilardi, 1976). Although it has been isolated from several human sources (Gilardi, 1972; Hugh & Gilardi, 1974; Debois *et al.*, 1975) it has only been implicated as a possible pathogen in two cases of chronic otitis media (Von Graevenitz & Simon, 1970), one of tibial wound infection (Gilardi, 1972) and one of infected chronic leg ulcers (Degreef, Debois & Vandepitte 1975; Debois *et al.*, 1975). This report emphasises the capability of *P. putrefaciens* strains to grow in the skin but fails to adduce conclusive evidence of pathogenicity in the lesions described. Organisms were isolated in mixed culture on every occasion, and all bacterial species were sensitive to cotrimoxazole and aminoglycosides *in vitro*. Response could have been due to a combination of hospitalization, proper nursing, treatment of cardiac failure, and disappearance of Gram-negative organisms following appropriate antimicrobial therapy. Sensitivity to streptomycin and gentamicin in disc diffusion tests probably reflects framycetin-sensitivity in all Gram-negative bacteria isolated (Lowbury & Ayliffe, 1974).

In contradistinction to *Pseudomonas aeruginosa*, *P. putrefaciens* is very susceptible to many commonly used antibiotics, including erythromycin, to which most Gram-negative bacteria are resistant (Debois *et al.*, 1975).

The affinity of *P. putrefaciens* for necrotic tissue with impaired blood supply has been described before (Debois *et al.*, 1975). In our patient, poor personal hygiene could have contributed to contamination of the ulcers with saprophytes from soil.

Pseudomonas putrefaciens must be borne in mind as a colonizer and possible pathogen in chronic ulcers on the extremities (especially the legs) of debilitated patients.

REFERENCES

- DEBOIS, J., DEGREEF, H., VANDEPITTE, J. & SPAEPEN, J. (1975) *Pseudomonas putrefaciens* as a cause of infection in humans. *Journal of Clinical Pathology*, **28**, 993.
- DEGREEF, H., DEBOIS, J. & VANDEPITTE, J. (1975) *Pseudomonas putrefaciens* as a cause of infection of venous ulcers. *Dermatologica*, **151**, 296.
- GILARDI, G.L. (1972) Infrequently encountered *Pseudomonas* species causing infection in humans. *Annals of Internal Medicine*, **77**, 211.
- GILARDI, G.L. (1976) *Pseudomonas* species in clinical microbiology. *Mount Sinai Journal of Medicine*, **43**, 710.
- HUGH, R. & GILARDI, G.L. (1974) *Pseudomonas*. In: *Manual of Clinical Microbiology* (Ed. by E.H. Lennette, E.H. Spaulding and J.P. Truant), 2nd edn, p.250. American Society for Microbiology, Washington D.C.
- LOWBURY, E.J.L. & AYLIFFE, G.A.J. (1974) Gram negative bacilli. In: *Drug Resistance in Antimicrobial Therapy* (Ed. by I.N. Kugelmass), p.118. Charles C. Thomas, Springfield.
- STOKES, E.J. (1975) *Clinical Bacteriology*, 4th edn, p.218. Edward Arnold, London.
- VON GRAEVENITZ, A. & SIMON, G. (1970) Potentially pathogenic, nonfermentative, H₂S—producing gram-negative rod (1b). *Applied Microbiology*, **19**, 176.

Leg ulcers in alpha-thalassaemia (haemoglobin H disease)

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SUMMARY

A case of recurrent leg ulceration associated with α -thalassaemia (haemoglobin H disease) is reported. It is suggested that leg ulcers occurring in the thalassaemic syndromes may be due to structural changes in the affected red cells which result in increased cell rigidity, decreased deformability and consequent diminished blood flow in capillary beds that are subjected to venous stasis.

Chronic leg ulcers are a well-known complication of certain hereditary haemolytic anaemias. They occur frequently in sickle-cell anaemia, and are well recognized in hereditary spherocytosis and thalassaemia (Wintrobe, 1974). In thalassaemia, leg ulcers have been described in both major and minor types of β -thalassaemia (Canor & Cohen, 1976), but have not been previously noted in α -thalassaemia.

We report here the case of a patient with α -thalassaemia intermedia (haemoglobin H disease), who has been admitted to hospital on three occasions in the last 12 years with leg ulcers.

CASE HISTORY

A 44-year-old Greek Cypriot cook was admitted to hospital with large ulcers above the right ankle. He had had recurrent ulceration around the right ankle since his early twenties. Clinical examination showed no evidence of varicose veins. He complained of shortness of breath on exertion and a slight cough. At the time of his admission he smoked between 20 and 40 cigarettes per day, but had smoked larger numbers of cigarettes in the past. A diagnosis of haemoglobin H disease had been made in 1968 during a hospital admission for leg ulceration, and at the time of a subsequent admission in 1972 he had been treated with a blood transfusion.

The family history was entirely negative. Both parents and five siblings were alive, and we were told that none had suffered from leg ulcers or anaemia. His three children had previously been screened for haemoglobin H disease and had been found to be normal.

Examination revealed three ulcers on the right leg—one over each of the malleoli and a third over the lower third of the tibia. The left leg showed no active ulceration, but pigmentation and scarring attributed to scalding from hot water during childhood were noted. The heart and lungs were normal and the spleen was not palpable on clinical examination. Slight icterus was noted.

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Haematological investigation showed: W.B.C. 7.4×10^9 cells/l. Total haemoglobin was 9.5 g/dl. P.C.V. 0.373. M.C.V. 72 fl. M.C.H. 18.3 pg. M.C.H.C. 25.1%. Reticulocytes 3.8%. A blood film showed normal white cell morphology, but the red cells showed marked anisocytosis, poikilocytosis, target cells and gross hypochromasia. Supravital staining with brilliant cresyl blue showed that all red cells contained numerous H-bodies. Haemoglobin electrophoresis revealed haemoglobins A and H at pH 8.4; haemoglobin H was confirmed at pH 6.5. Haemoglobin H comprised 23.5% of total haemoglobin. Carboxyhaemoglobin comprised 7.4% of total haemoglobin. Glucose-6-phosphate-dehydrogenase (G6PD) was raised at 388 mU/ 10^9 RBCs (normal range 120–140 mU/ 10^9 RBCs), and this raised level was attributed to the reticulocytosis. Plasma bilirubin was raised at 40 μ mol/l, but plasma viscosity, urea and electrolytes, plasma albumin, plasma globulin, plasma aspartate aminotransferase, serum iron and total iron binding capacity, serum and red cell folate, leukocyte ascorbic acid, and plasma zinc levels were all within normal limits.

Radiological examination of the right leg showed porotic changes with periosteal new bone formation in areas underlying the leg ulcers. A venogram showed completely normal superficial and deep veins.

The patient's leg ulceration was treated by bed rest, and Eusol and paraffin dressings. He was strongly advised to stop smoking. After 4 weeks' treatment the leg ulcer had almost completely healed.

DISCUSSION

Normal human haemoglobins are of three types—Adult (Hb A), fetal (Hb F), and Hb A₂.

The globin molecule of each of these consists of four subunits—two α -polypeptide chains and two other polypeptide chains which are identified by a Greek letter. Hb A, with two alpha chains and two beta chains ($\alpha_2\beta_2$) makes up about 98% of total haemoglobin in the adult, while Hb A₂ ($\alpha_2\delta_2$) makes up the remaining 1–3%. Hb F ($\alpha_2\gamma_2$), which accounts for 50% to 70% of haemoglobin present in the newborn, rapidly diminishes to less than 2% by early infancy.

The thalassaemias comprise a heterogeneous group of inherited disorders of haemoglobin synthesis which have one common denominator—a decreased rate of synthesis of one or more of the normal polypeptide chains. Modern classification of these disorders depends on which polypeptide chains are deficient. In the more common beta-thalassaemia, alpha chain synthesis is normal but beta chains are deficient. As a result, Hb A is produced in small amounts, and excess alpha chains join with gamma or delta chains to form increased amounts of Hb F ($\alpha_2\gamma_2$) and Hb A₂ ($\alpha_2\delta_2$). Clinical severity varies greatly and cases are classified further into a large number of sub-groups—depending on haemoglobin pattern, clinical features and genotype (Weatherall & Clegg, 1972).

The alpha-thalassaemic syndromes are the result of inadequate synthesis of alpha chains, and therefore the production of all three haemoglobins, i.e. Hb A, Hb F and Hb A₂, is affected. Surplus gamma chains, produced in fetal life, unite to give a tetrameric haemoglobin, Hb Barts (γ_4), which may be detected in neonates, but in older children and in adults this is replaced by Hb H (β_4) formed as a result of surplus beta chains. Alpha-thalassaemia may present with four degrees of severity, but only two of these are of clinical significance. In the Hb Barts hydrops fetalis syndrome, death of the fetus occurs, but in Hb H disease—which is particularly prevalent in South East Asia, the Middle East, Greece and Cyprus—clinical severity is variable and most patients present a picture of mild haemolytic anaemia.

In Hb H disease, Hb H makes up from 4 to 30% of the total haemoglobin with decreased levels of Hb A₂, and the blood film shows poikilocytosis, microcytosis, hypochromia, target cells and a number of tiny mis-shapen red cells. Hb H is thermolabile and unstable. It has an affinity for oxygen

ten times that of Hb A, but gives up its oxygen less readily at low oxygen tensions, thus aggravating the effect of the anaemia. Hb H is easily oxidized and then precipitates in the red cell forming inclusion bodies. These may be demonstrated by incubating the cells with 1% brilliant cresyl blue. It is thought that the extreme degree of poikilocytosis seen in Hb H disease is due to the removal of the rigid inclusion bodies from the red cells as they pass through the reticulo-endothelial system and spleen. This may also result in cells of bizarre shape, or the splitting of cells into two or more fragments (Wennberg & Weiss, 1968). The cells of β -thalassaemia major are known to be unduly rigid and less deformable than normal because of their intracellular inclusions (Zaino & Rossi, 1974) and it seems likely that similar but less severe changes occur in Hb H disease.

The cause of leg ulceration in patients with β -thalassaemia is unknown. Factors such as anaemia, venous insufficiency and infection may play a part, but the increased incidence of ulceration in this condition suggests that some other factor is also important. Peachey (1978), in an attempt to explain the ulceration, has suggested that the increased cell rigidity and decreased deformability known to occur in thalassaemic red cells may be a cause of diminished blood flow in capillary beds subjected to venous stasis and, thus, predispose affected subjects to leg ulceration.

Leg ulcers do not usually occur in Hb H disease, and this may be because the red cell changes are less severe than in β -thalassaemia. In the present case the relatively high percentage of Hb H and the patient's heavy smoking habits may have been enough, in combination with other factors such as venous stasis, to tip the balance in favour of ulceration.

REFERENCES

- CANOR, S. & COHEN, T. (1976) Leg ulcers in a family with both beta-thalassaemia and glucose 6 phosphate dehydrogenase deficiency. *British Journal of Dermatology*, **95**, 203.
- PEACHEY, R.D.G. (1978) Leg ulceration and haemolytic anaemia: an hypothesis. *British Journal of Dermatology*, **98**, 245.
- WEATHERALL, D.J. & CLEGG, J.B. (1972) *The Thalassaemia Syndromes*, 2nd edn. Blackwell Scientific Publications, Oxford.
- WENNBERG, E. & WEISS, L. (1968) Splenic erythroclasia: an electron microscopic study of haemoglobin H disease. *Blood*, **31**, 778.
- WINTROBE, M.M. (1974) *Clinical Haematology*, 7th edn., p. 723. Lea and Febinger, Philadelphia.
- ZAINO, E.C. & ROSSI, M.B. (1974) Ultrastructure of the erythrocytes in beta-thalassaemia. *Annals of the New York Academy of Sciences*, **232**, 238.

Pathogenicity of the intercellular antibodies of pemphigus and their periodic removal from the circulation by plasmapheresis

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SUMMARY

The authors are convinced that the intercellular antibodies of pemphigus are pathogenic. Consequently periodic plasmapheresis in two patients with pemphigus has been performed to remove the antibodies from the circulation. After larger plasma-exchanges there was a decrease of the antibody titre and a parallel improvement of the clinical condition.

We are convinced that the intercellular antibodies of pemphigus are pathogenic for the following reasons:

In vitro. (a) Whole pemphigus serum (Bellone & Leone, 1956), or the purified fraction of pemphigus IgG (PIgG) alone in the absence of complement (Schiltz & Michel, 1976; Beutner, Jordan & Chorzelski, 1976), is able to produce epidermal acantholysis in normal human skin in organ cultures (Fig. 1). The fixation of intercellular antibodies on the intercellular spaces precedes the acantholysis by some hours (Schiltz & Michel, 1976).

(b) The acantholytic cells cultured *in vitro* with normal serum regain normal maturation but the addition of pemphigus serum causes persistence of the acantholytic changes (Mele & Nazzaro, 1955; Pisani *et al.*, 1977) (Fig. 2). If it is true that the pemphigus serum is unable to produce acantholytic changes in normal epidermal cells cultured *in vitro* (Pisani *et al.*, 1977), it is certain, as demonstrated by immunofluorescent staining, that it contains an antibody that recognizes a membrane receptor, stable in tissue culture (Ruocco *et al.*, 1977a).

In vivo. (a) A few cases have been described of burns evolving into true pemphigus; circulating intercellular antibodies at an unusually high titre were found (Chorzelski *et al.*, 1971; Chorzelski, 1974; Beutner *et al.*, 1976).

(b) A case of giant follicular lymphoma has been reported, characterized by a clone of lymphoid tissue producing intercellular antibodies, and evolving into true pemphigus (Saikia, 1972).

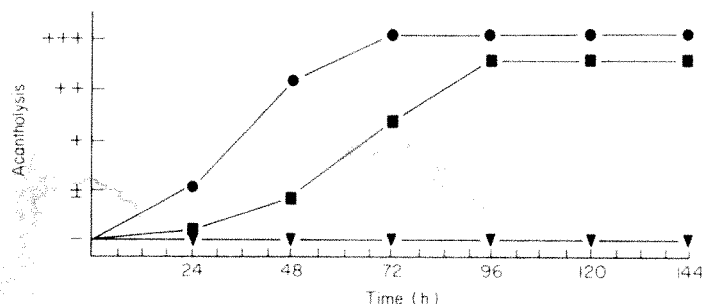


FIGURE 1. Production of epidermal acantholysis in normal human skin cultured *in vitro*. (●) Normal human skin with addition of PIgG; (■) normal human skin with addition of whole pemphigus serum; (▲) normal human skin with addition of normal human serum.

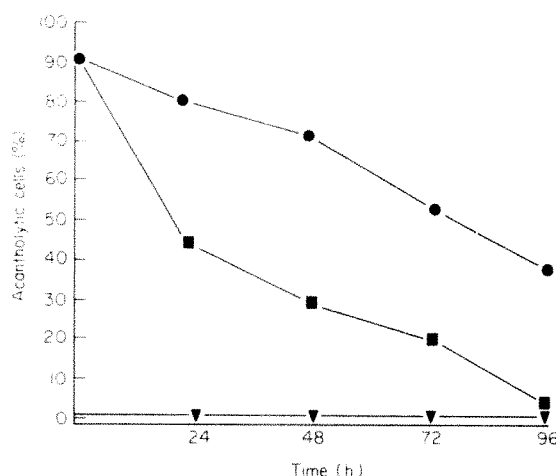


FIGURE 2. Pattern of acantholytic changes in human epidermal cells cultured *in vitro*. (●) Pemphigus epidermal cells cultured with addition of pemphigus serum (10%); (■) pemphigus epidermal cells cultured with addition of normal human serum (10%); (▲) normal epidermal cells cultured with addition of pemphigus serum (10%).

(c) Newborn infants of mothers affected with active pemphigus frequently have a bullous eruption not lasting more than 2 weeks (Blumenthal, 1908; Werther, 1925; Kolonja, 1952; Hofmann & Cainelli, 1962; Ruocco *et al.*, 1975). Similarly acantholytic cells with deposits of IgG on the membrane have been found by Ruocco *et al.* in the bulla of a newborn infant. This is attributed to trans-placental transfer of PIgG (Ruocco *et al.*, 1975). This 'experiment of nature' strongly confirms the pathogenic role of these antibodies. We therefore considered it logical to perform periodic plasmapheresis in order to remove the PIgG from the circulation as a treatment for pemphigus.

So far our experience is limited to two patients with pemphigus vulgaris of average severity (Ruocco *et al.*, 1977c) in whom it was necessary to suspend corticosteroid treatment because of complications. Bearing in mind the 2-3 week half-life of the antibodies, plasmapheresis was performed (4 times for each patient) at about 10 day intervals. The plasma taken from the patient was substituted with equal quantities of donors' plasma, half fresh and half stored. The fresh plasma is necessary to replace the labile coagulation factors. According to the availability of fresh plasma we carried out plasma-exchanges of 2000-4000 ml. The antibody titre (Figs 3 and 4) was frequently tested on guinea-pig and monkey oesophagus.

TABLE 1. Clinical aspects of the pathogenicity of pemphigus antibodies

Biological status	Clinical status	Immunofluorescent staining for anti-ICS antibody	Tzanck's test	Clinical evolution
Skin damage	(a) 3 cases	DS++ (persistent)	++	Pemphigus
	Burns	IS++		
	(b) most cases	DS± (temporary)	—	—
		IS±		
Production of clonal IgG	Lymphoma (1 case)	DS++	++	Pemphigus
		IS++		
	IgG-myeloma* (3 cases)	DS—	—	—
		IS—		
Transplacental 'PlgG' (?) in newborn infants	(a) 1 case	DS++†	++†	Regression after 2 weeks
	Transient neonatal pemphigus	IS n.d.	n.d.	Regression after 2 weeks
	(b) 5 cases	DS n.d.		
Transplacental 'normal' IgG in newborn infants	Healthy newborn infants (7 cases)*	DS—	—	—
		IS—		

* Our unpublished data.

† Negative after 2 weeks.

n.d. = not done; DS = direct staining; IS = indirect staining.

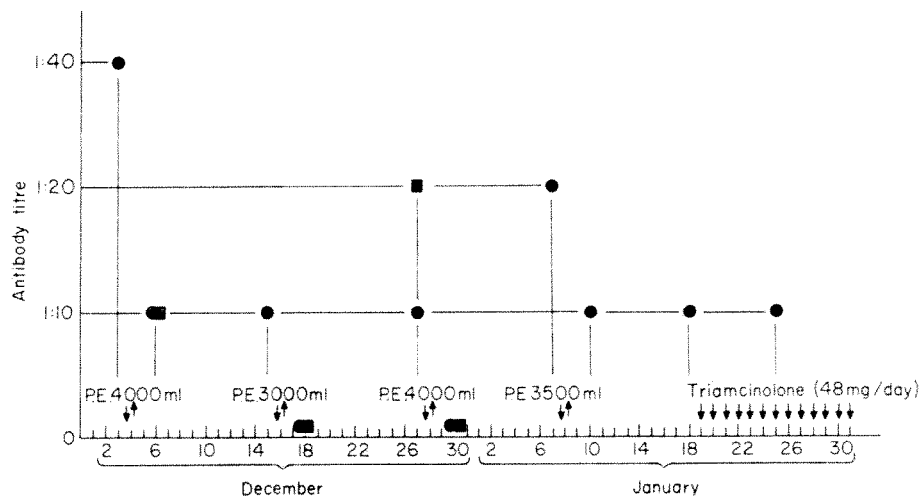


FIGURE 3. Case 1. Repeated anti-ICS (intercellular substance) antibody determinations by indirect immunofluorescence staining related to periodic plasmapheresis. Marked decrease of titre after 3000-4000 ml plasma-exchange. Fixed titre with steroid therapy. (●) Guinea-pig oesophagus; (■) monkey oesophagus; P.E., plasma exchange.

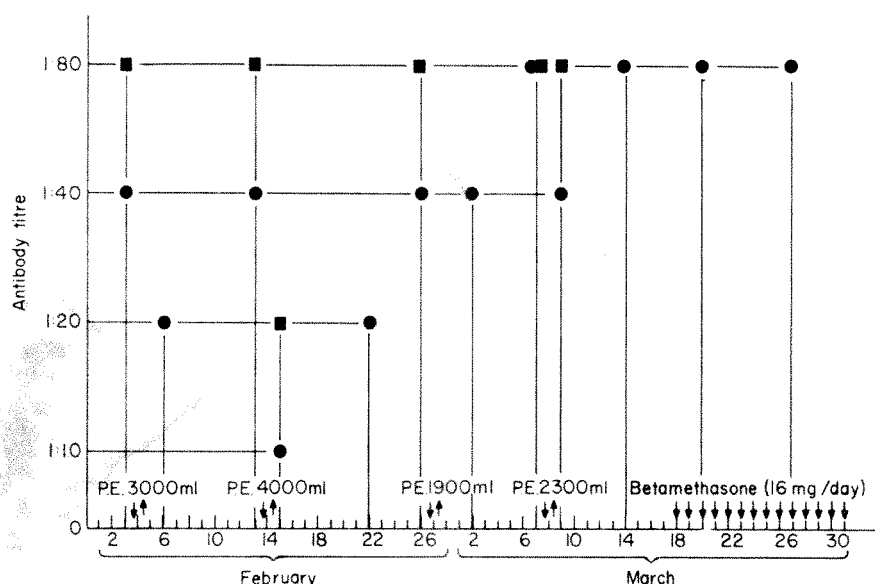


FIGURE 4. Case 2. Repeated anti-ICS antibody determinations by indirect immunofluorescence staining related to periodic plasmapheresis. Marked decrease of titre *only* after larger plasma-exchanges. Progressive increase in the intervals. (●) Guinea-pig oesophagus; (■) monkey oesophagus; P.E., plasma exchange.

When it was possible to make larger exchanges there was a decrease of the antibody titre and parallel improvement of the clinical condition. Also the Tzanck test showed reduction of the acantholytic changes after the fourth plasmapheresis.

In our patients plasmapheresis had to replace corticosteroids temporarily. However, we believe that in other cases treatment with plasmapheresis could be even more efficient if integrated with steroid and immunosuppressive therapy. We outline the following proposals for treatment:

- (1) Removal from the circulation—by means of massive plasmapheresis—of the largest possible quantity of PIgG.
- (2) Concomitant administration of systemic steroids, and later other immunosuppressive drugs, to slow down the synthesis of new PIgG.
- (3) Careful monitoring of the antibody titre by indirect immunofluorescence.
- (4) Recourse to further plasmapheresis should the titre increase to critical levels.

This treatment resembles that successfully carried out recently in autoimmune nephritis at The Hammersmith Hospital, London (Lockwood *et al.*, 1977).

REFERENCES

- BELLONE, A.G. & LEONE, V. (1956) Ricerche sull'influenza esercitata da sieri di soggetti sani o affetti da pemfigo su pelle umana normale e pemfigosa coltivata in vitro. *Giornale Italiano di Dermatologia e Sifilografia*, **97**, 97.
- BEUTNER, E.H., JORDAN, R.E. & CHORZELSKI, T.P. (1976) Autosensitization in pemphigus, bullous pemphigoid and other chronic bullous diseases. In: *Textbook of Immunopathology* (ed. by P.A. Miescher and H.J. Müller-Eberhard), Vol. 2, p. 931. Grune & Stratton, New York.
- BLUMENTHAL, F. (1908) Ein intrauterin entstandenes toxisches bullöses Erythem. *Archiv für Dermatologie*, **93**, 43.
- CHORZELSKI, T.P., JABLONSKA, S., BEUTNER, E.H. & KOWALSKA, M. (1971) Can pemphigus be provoked by a burn? *British Journal of Dermatology*, **85**, 320.

- CHORZELSKI, T.P. (1974) Clinical aspects of the pathogenicity of pemphigus antibodies. In: *Dermatology* (Ed. by F. Flarer, F. Serri and D.W.K. Cotton), p. 77. Excerpta Medica, Amsterdam.
- HOFMANN, F. & CAINELLI, T. (1962) Pemfigo, gravidanza e terapia corticosteroidea. *Giornale Italiano di Dermatologia*, **75**, 193.
- KOLONJA, S. (1952) Pemphigus und Schwangerschaft. *Zentralblatt für Gynäkologie*, **74**, 1831.
- LOCKWOOD, C.M., PINCHING, A.J., SWENY, P., REES, A.J., PUSSELL, B., UFF, J. & PETERS, D.K. (1977) Plasma-exchange and immunosuppression in the treatment of fulminating immune-complex crescentic nephritis. *Lancet*, **i**, 63.
- MELE, V. & NAZZARO, P. (1955) Ricerche di citologia sperimentale. Relazione sul Pemfigo. *Minerva Dermatologica* — *Atti SIDES*, suppl. **30**, 449.
- PISANI, M., RUOCO, V., VILLANO, P.A., GORGA, F. & ROMANO, C. (1977) Colture *in vitro* di epitelociti pemfigosi: persistenza e regressione dei citodismorfismi acantolitici. *Minerva Dermatologica*, in press.
- RUOCO, V., PISANI, M., GORGA, F., ALVIGGI, L. & ASTARITA, C. (1977a) Colture *in vitro* di epitelociti di soggetti sani con aggiunta di siero pemfigoso: reperti in immunofluorescenza. *Minerva Dermatologica*, in press.
- RUOCO, V., ROSSI, A., ASTARITA, C., ALVIGGI, L. & CATALANO, G. (1975) A congenital acantholytic bullous eruption in the new-born infant of a pemphigous mother. *Italian General Review of Dermatology*, **12**, 169.
- RUOCO, V., ROSSI, A., ARGENZIANO, G., ASTARITA, C., ALVIGGI, L., FARZATI, B. & PAPALEO, G. (1977b) Originale trattamento del pemfigo volgare con plasmaferesi periodiche. *Minerva Dermatologica*, in press.
- SAIKIA, N.K. (1972) Extraction of pemphigus antibodies from a lymphoid neoplasm and its possible relationship to pemphigus vulgaris. *British Journal of Dermatology*, **86**, 411.
- SCHILTZ, J.R. & MICHEL, B. (1976) Production of epidermal acantholysis in normal human skin *in vitro* by the IgG fraction from pemphigus serum. *Journal of Investigative Dermatology*, **67**, 254.
- WERTHER, J. (1925) Beobachtungen über Pemphigus vulgaris chronicus (drei Fälle bei Schwangerschaft, zwei Fälle von Pemphigus vulgaris congenitus). *Dermatologische Wochenschrift*, **81**, 1334.

Brief Communication

Plasma exchange in the treatment of pemphigus vulgaris

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The technique of plasma exchange has been applied to a number of immune disorders in which circulating antibodies are present (Lockwood *et al.*, 1976; Pinching *et al.*, 1976). The serum of patients with pemphigus vulgaris contains an antibody (usually IgG) directed against an antigen in the epidermal intercellular spaces. There is some evidence that the titre of this antibody correlates with disease activity (Sams & Jordon, 1971). It seemed that plasma exchange, with removal of the circulating antibody, might be a useful adjunct to conventional treatment.

The patient, a 49-year-old man, presented in December 1975. The clinical diagnosis of pemphigus vulgaris was confirmed by histological and immunohistological examination of a skin biopsy. The disease was controlled with systemic prednisolone, but in the subsequent 18-month period it proved impossible to reduce the daily dose below 15 mg without precipitating a relapse. The development of serious steroid side effects appeared inevitable.

There were no contra-indications to plasmapheresis and informed consent to the procedure was obtained. Three plasma exchanges were carried out during a 2-week period using a Haemonetics Model 30 cell separator (Haemonetics Corporation, Natick, Boston, U.S.A.). Volumes ranging from 3.3 to 3.6 litres were exchanged for fresh frozen plasma. Forearm veins were used for vascular access. On the whole the procedure was well tolerated.

Pemphigus antibodies were present in a titre of 1/45 prior to plasma exchange. Thereafter they remained undetectable during the 3-week study period. The presence of inter-epidermal cell antibodies was confirmed by skin biopsy before exchange in lesions and uninvolved skin. Biopsy of uninvolved skin after the exchanges did not demonstrate antibodies but this fact must be cautiously interpreted since the distribution of these antibodies in unaffected skin may well be patchy.

Unfortunately, there was no demonstrable clinical benefit from the procedure. New blisters formed throughout the period of treatment. Plasma exchange was terminated because of difficulties with vascular access.

Despite our lack of success we propose to re-evaluate this form of treatment in other suitable patients. If applied to new cases with high titres of circulating antibodies, control of the disease might be obtainable with less than the present formidable doses of corticosteroids.

Recently, plasma exchange has proved of use in the management of herpes gestationis (Carruthers & Ewins, 1978), a disease in which clinical activity does not correlate with the plasma levels of any recognized factor.

REFERENCES

- CARRUTHERS, J.A. & EWINS, A.R. (1978) *Clinical & Experimental Immunology*, (in press).
LOCKWOOD, C.M., REES, A.J., PEARSON, T.A., EVANS, D.J., PETERS, D.K. & WILSON, C.B. (1976) Immunosuppression and plasma exchange in the treatment of Goodpasture's syndrome. *Lancet*, **i**, 711.
PINCHING, A.J., PETERS, D.K. & NEWSOME DAVIS, J. (1976) Remission of myasthenia gravis following plasma exchange. *Lancet*, **ii**, 1373.
SAMS, W.M. & JORDON, R.E. (1971) Correlation of pemphigoid and pemphigus antibody titres with activity of disease. *British Journal of Dermatology*, **84**, 7.

Comment

LEG ULCERATION AND HAEMOLYTIC ANAEMIA:
AN HYPOTHESIS

Leg ulceration is a well recognized complication of sickle-cell anaemia, β -thalassaemia and hereditary spherocytosis (Wintrobe, 1974). In addition, it has also been reported rarely in hereditary elliptocytosis (Hunter & Adams, 1929), pyruvate kinase deficiency (Tanaka & Paglia, 1971), α -thalassaemia (Daneshmend & Peachey, 1978), thrombocytopenic purpura (Witts, 1942) and in patients with splenomegaly due to other conditions (Gendel, 1948), but in some of these cases it is possible that the association may have been coincidental. With the exception of sickle-cell anaemia, the cause of the leg ulceration is poorly understood. The purpose of the present review is to discuss recent advances in our understanding of the three main conditions, and in the light of these findings to put forward an hypothesis to explain the associated leg ulceration.

Chronic leg ulcers occur in between 50 and 75% of older children and adults with homozygous sickle-cell anaemia. The incidence in patients with combination haemoglobinopathies is lower. In Jamaica it has been shown that the sickle-cell trait is significantly commoner in patients with leg ulcers than would be expected from the incidence of the trait in the Negro population (Serjeant & Gueri, 1970). Occasional patients with sickle-cell trait develop ulceration in the presence of associated systemic disease, such as pneumonia (Wolfort & Krizek, 1969).

Adult human haemoglobin (Hb A) consists of four sub-units—a pair of α and a pair of β polypeptide chains (Braunitzer *et al.*, 1964)—to each of which is attached a haem group. Haemoglobin S is formed as a result of the substitution of valine for glutamic acid at the sixth residue from the N terminal end of the β polypeptide chain of haemoglobin. Cells assume the sickle shape when the haemoglobin they contain is deprived of oxygen. This change may begin within ten seconds of deoxygenation, being favoured by a lowering of pH. Under these conditions, sickle haemoglobin gels and polymerizes to give rod-like structures consisting of six monomolecular strands of haemoglobin S twisted into a spiral. The rods aggregate into bundles to form tactoids, which lie parallel to the long axis of the cell or project into the projecting spicules (White & Heagan, 1973; White, 1974). It is believed that tactoid formation within the cell causes deformity of the cell membrane and the changes of sickling, but there is also evidence to suggest that the state of the cell membrane can influence the configuration assumed by the polymers (White & Heagan, 1973). Reversion to a normal shape occurs rapidly on re-oxygenation of the cell, but after repeated sickling, cells may lose their ability to revert to normal and remain irreversibly sickled. Even though the haemoglobin in these cells may be in the unpolymerized state, the cell membrane—due to ATP depletion—accumulates calcium and becomes rigid and misshapen (Bertles & Döbler, 1969).

Recent work has shown that erythrocyte deformability is a major factor determining blood viscosity (Chien *et al.*, 1967a). In sickle-cell anaemia deoxygenation causes a marked rise in whole blood viscosity. This occurs as a result of increase in the internal viscosity of the individual cells, while in addition the sickled cells are relatively rigid and unable to alter their shape (Chien, Usami & Bertles, 1970). These changes may cause a gross reduction of blood flow within certain capillary beds. If blood flow through the bed is rapid, the time of exposure of the erythrocyte to hypoxia may be too short for sickling to occur, but in capillary beds subjected to venous stasis the time factor and low oxygen tensions act to the detriment of the erythrocyte (Gabuzda, 1975).

In the thalassaemic syndromes it is now known that there is a deficiency of one or other of the normal polypeptide chains, which combine to form the haemoglobin molecule. Thus in β -thalassaemia

there is decreased synthesis of β chains and in α -thalassaemia a decrease in α chain production (Weatherall & Clegg, 1972). Leg ulcers are common in severe β -thalassaemia, but rare in α -thalassaemia (Daneshmend & Peachey, 1978).

In β -thalassaemia major the red cells are thin, distorted in shape, variable in size and contain little haemoglobin. Electron microscopy has shown gross distortion and deformity of the cells, with prominent infolding of the plasma membrane and vacuole formation. Iron may be found in different forms within membrane bound particles and mitochondria, and Heinz body-formation is prominent (Polliack & Rachmilewitz, 1973). Marked alterations in surface texture of the cells have also been noted (Hoffman *et al.*, 1956). It has been shown that the deficiency of β chain synthesis results in an excess of α chains within the cell (Modell *et al.*, 1969; Yataganas, Fessus & Gahrton, 1972). Some of these combine with newly made β and γ chains, but the remainder tend to precipitate and become associated with the cell membrane forming inclusion bodies. These inclusion bodies and, in particular, the rigid α chain precipitates make the thalassaemic cell rigid and less deformable. As a result of these changes damage to or destruction of the cell is more likely as it passes through the narrow constrictions of the red pulp of the spleen—where the ability of the red cell to deform is essential to its survival (Zaino & Rossi, 1974). Similar intracellular precipitates are uniformly, though minimally, present in thalassaemia minor. It is probable that small amounts of precipitated haemoglobin may be 'pitted' from the cell by the action of the spleen, but that if the inclusions are more pronounced the cell becomes more rigid and distorted, and its life span is shortened (Zaino & Rossi, 1974).

In hereditary spherocytosis (H.S.) there is a variable degree of anaemia, splenomegaly, spherocytosis and increased osmotic fragility of the red cells. The mean red cell volume may be normal, increased or decreased, but the red cell diameter is reduced as a result of the cell being spherocytic in shape rather than biconcave. Normal red cells are 2.0 μm or less in thickness, but in H.S. red cell thickness varies from 2.2–3.4 μm , and in typical cases red cell osmotic fragility is increased (Wintrobe, 1974). The vulnerability of these cells to destruction, especially in the spleen, accounts for the haemolytic anaemia, although the effects of this may be, in part, compensated by increased erythropoiesis.

The cell membrane of the normal red cell maintains active cation transport, whereby a high concentration of K is maintained within the cell and Na is pumped out (Hoffman, 1966). The mechanism which regulates the electrolytic relationship within the cell also regulates intracellular volume, and if the active cation transport mechanism is impaired the cell accumulates sodium and water and eventually haemolyses. This transport system is dependent on the cell membrane associated enzyme ATPase (Katz & Epstein, 1968) and needs a continuous supply of glucose for its efficient function. Lack of glucose, such as may occur in conditions of red cell sequestration, leads to depletion of red cell ATP and to a loss of normal membrane impermeability.

In hereditary spherocytosis there is an increased rate of influx of Na into the cell (Wiley, 1972). This results in stimulation of the ATPase system which, in providing energy to pump the Na out of the cell, liberates ADP and inorganic phosphate, and stimulates glycolysis. Because of the stimulation of glycolysis, spherocytic red cells are especially vulnerable to the effects of sequestration. Although it was at one time thought that these changes were the cause of haemolysis in H.S., Wiley (1970) has shown that there is an inverse correlation between the survival of spherocytic red cells and their cation permeability—the 'leakiest' cells having the longest survival *in vivo*.

As a result of recent work, it has become increasingly obvious that a membrane defect is important in the pathophysiology of H.S. There is evidence that red cells in H.S. lose membrane lipid when incubated for a few hours *in vitro* (Reed & Swisher, 1966; Cooper & Jandl, 1969), and that the cells that haemolyse are those that have lost a lot of membrane lipid with a resultant decrease in their surface area/volume ratio. After splenectomy (Langley & Felderhof, 1968) red cell survival becomes

almost normal, and the amount of membrane lipid per cell returns towards normal, but the defect in the cation transport system persists.

Jandl, Simmons & Castle (1961) and Murphy (1967) have emphasized the greatly decreased deformability of spherocytes in H.S., and Leblond, LaCelle & Weed (1971) have shown that this decreased deformability develops during the life span of the red cell in the peripheral circulation. Weed, LaCelle & Merrill (1969) put forward evidence to show that ATP is of major importance in the preservation of normal red cell membrane deformability, depletion of intracellular ATP being associated with a large increase in red cell calcium and greatly decreased membrane deformability. The exact nature of the membrane deficit in H.S. still awaits elucidation, but it is now believed that the abnormal rigidity and decreased deformability of the red cell are of major importance and that, as a result, cells become trapped in the splenic pulp with consequent sequestration and haemolysis (Weed, 1975).

Blood flow to the skin is distributed by a candelabra pattern of vertically orientated arterioles, which break up into capillaries. Normal dermal papillae are supplied by individual papillary capillaries—with an internal diameter of approximately $15\ \mu$ (Ryan, 1973)—which loop up into the papillae and then empty into the horizontal sub-papillary venous plexus. It has been shown (Ryan, 1973) that the papillary capillaries in areas of relative venous stasis are unduly elongated, coiled and dilated. Ryan (1969) has observed that blood collects in the venous end of the loop and only intermittently empties into the horizontal venous plexus, and believes that this is due to a normal and variable degree of obstruction to outflow from the venous end of the loop. Minor degrees of congestion in the papillary vessels encourage shunting of blood, so that the papillary vessel is temporarily bypassed and blood passes direct from the arteriole into the horizontal venous plexus. In the presence of venous incompetence this effect is accentuated, and the cause of venous ulceration is probably a combination of severe congestion leading to cessation of blood flow and short-circuiting which tends to deprive the whole epidermis of adequate nourishment.

In patients with β -thalassaemia or H.S. the cause of leg ulceration is poorly understood. Trauma, anaemia, venous insufficiency and infection may all play a part in affected patients, but the increased incidence of leg ulcers in these diseases suggests that some other factor is particularly important. Normal red cells are pliable and easily deformable, and it has been shown that the ability of the erythrocyte to deform is a major determinant of flow in narrow capillary vessels (Wells, 1964). In β -thalassaemia and H.S. the red cells are unduly rigid and show reduced deformability, while similar changes occur in the red cells of patients with sickle-cell anaemia under conditions of oxygen lack. A recent review of the clinical implications of alteration in red cell shape (Lessin, King & Jensen, 1976) emphasizes the fact that sickled cells, spherocytes and Heinz body-containing erythrocytes all cause an increase in blood viscosity—particularly at low (capillary) shear rates. In all three conditions the change in the red cell membrane and the increased rigidity of the cell are thought to be a major factor leading to sequestration of red cells in the spleen and subsequent haemolysis. In H.S. there is some evidence to suggest that passage through the spleen is, in part, a cause of the red cell membrane abnormality, and splenectomy results in a reduction of the membrane changes and a marked increase in cell survival. In addition, leg ulceration is usually improved.

In sickle-cell anaemia, hereditary spherocytosis and β -thalassaemia leg ulcers occur, as do pure venous ulcers, in the gaiter area, and it seems likely that venous stasis at least plays a part in determining the site of ulceration. In the same way that the abnormally shaped, rigid and poorly deformable cells do not pass easily through the small constricted spaces of the spleen, it is likely that these cells would be less easily cleared than normal cells from the papillary capillaries and, particularly, from the relatively congested and obstructed venous end of the capillary loop in areas of mild venous stasis. Chien *et al.* (1967b) have shown that in conditions of red cell overcrowding, such as occurs at the

venous end of the loop, the ability of the cells to deform assumes increasing importance if blood flow is to occur. In each of these three conditions the red cell abnormality might be expected to accentuate any stasis and congestion in the papillary vessels of the lower leg of predisposed patients and, if this became severe enough, subsequent short-circuiting of the blood flow would be likely to result in impaired skin nutrition and an eventual tendency to ulceration in the gaiter area.

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R.D.G. PEACHEY

REFERENCES

- BERTLES, J.F. & DÖBLER, J. (1969) Reversible and irreversible sickling: a distinction by electron microscopy. *Blood*, **33**, 884.
- BRAUNITZER, G., HILSE, K., RUDLOFF, V. & HILSCHMANN, N. (1964) The hemoglobins. *Advances in Protein Chemistry*, **19**, 1.
- CHIEN, S., USAMI, S., DELLENBACK, R.J. & GREGERSEN, M.I. (1967a) Blood viscosity: influence of erythrocyte deformation. *Science*, **157**, 827.
- CHIEN, S., USAMI, S., DELLENBACK, R.J. & GREGERSEN, M.I. (1967b) Blood viscosity: influence of erythrocyte aggregation. *Science*, **157**, 829.
- CHIEN, S., USAMI, S. & BERTLES, J.F. (1970) Abnormal rheology of oxygenated blood in sickle-cell anaemia. *Journal of Clinical Investigation*, **49**, 623.
- COOPER, R.A. & JANDL, J.H. (1969) The role of membrane lipids in the survival of red cells in hereditary spherocytosis. *Journal of Clinical Investigation*, **48**, 736.
- DANESHMEND, T.K. & PEACHEY, R.D.G. (1978) Leg ulcers in α -thalassaemia (Haemoglobin H disease). *British Journal of Dermatology*, **98**, 233.
- GABUZDA, T.G. (1975) Sickle-cell leg ulcers. *International Journal of Dermatology*, **14**, 322.
- GENDEL, B.R. (1948) Chronic leg ulcer in diseases of the blood. *Blood*, **3**, 1283.
- HOFFMAN, J.F., WOLMAN, I.J., HILLIER, J. & PARPART, A.K. (1956) Ultrastructure of erythrocyte membranes in thalassaemia major and minor. *Blood*, **11**, 946.
- HOFFMAN, J.F. (1966) The red cell membrane and the transport of sodium and potassium. *American Journal of Medicine*, **41**, 666.
- HUNTER, W.C. & ADAMS, R.B. (1929) Haematologic study of three generations of a 'white' family showing elliptical erythrocytes. *Annals of Internal Medicine*, **2**, 1163.
- JANDL, J.H., SIMMONS, R.L. & CASTLE, W.B. (1961) Red cell filtration and the pathogenesis of certain haemolytic anaemias. *Blood*, **18**, 133.
- KATZ, A.I. & EPSTEIN, F.H. (1968) Physiologic role of sodium-potassium-activated adenosine triphosphatase in the transport of cations across biologic membranes. *New England Journal of Medicine*, **278**, 253.
- LANGLEY, G.R. & FELDERHOF, C.H. (1968) Atypical autohaemolysis in hereditary spherocytosis as a reflection of two cell populations: relationship of cell lipids to conditioning by the spleen. *Blood*, **32**, 569.
- LEBLOND, P.F., LACELLE, P.L. & WEED, R.I. (1971) Rhéologie des érythroblastes et des érythrocytes dans la sphérocytose congénitale. *Nouvelle Revue Française d'Hématologie*, **11**, 537.
- LESSIN, L., KING, P. & JENSEN, W. (1976) Clinical implications of red cell shape. In: *Advances in Internal Medicine* (Ed. by G. H. Stollerman), Vol. 21, p. 451. Year Book Medical Publishers, Chicago.
- MODELL, C.B., LATTER, A., STEADMAN, J.H. & HUEHNS, E.R. (1969) Haemoglobin synthesis in β -thalassaemia. *British Journal of Haematology*, **17**, 485.
- MURPHY, J.R. (1967) The influence of pH and temperature on some physical properties of normal erythrocytes and erythrocytes from patients with hereditary spherocytosis. *Journal of Laboratory and Clinical Medicine*, **69**, 758.
- POLLACK, A. & RACHMILEWITZ, E.A. (1973) Ultrastructural studies in β -thalassaemia major. *British Journal of Haematology*, **24**, 319.
- REED, C.F. & SWISHER, S.N. (1966) Erythrocyte lipid loss in hereditary spherocytosis. *Journal of Clinical Investigation*, **45**, 777.
- RYAN, T.J. (1969) The epidermis and its blood supply in venous disorders of the leg. *Transactions of St John's Hospital Dermatological Society*, **55**, 51.

- RYAN, T.J. (1973) Structure, pattern and shape of blood vessels of the skin. In: *The Physiology and Pathophysiology of the Skin* (Ed. by A. Jarrett), Vol. 2, Ch. 16. Academic Press, London.
- SERGEANT, G.R. & GUERI, M. (1970) Sickle-cell trait and leg ulceration. *British Medical Journal*, **i**, 820.
- TANAKA, K.R. & PAGLIA, D.E. (1971) Pyruvate kinase deficiency. *Seminars in Haematology*, **8**, 367.
- WEATHERALL, D.J. & CLEGG, J.B. (1972) *The Thalassaemia Syndromes*, 2nd edn. Blackwell Scientific Publications, Oxford.
- WEED, R.I., LACELLE, P.L. & MERRILL, E.W. (1969) Metabolic dependence of red cell deformability. *Journal of Clinical Investigation*, **48**, 795.
- WEED, R.I. (1975) Hereditary spherocytosis: a review. *Archives of Internal Medicine*, **135**, 1316.
- WELLS, R.E. JR. (1964) Rheology of blood in the microvasculature. *New England Journal of Medicine*, **270**, 832.
- WHITE, J.G. & HEAGAN, B. (1973) Fine structure of haemoglobin polymerisation. In: *Sickle-Cell Disease: Diagnosis, Management, Education and Research* (Ed. by H. Abramson, J. F. Bertles and D. L. Wethers), p. 104. The C. V. Mosby Company, St. Louis.
- WHITE, J.G. (1974) Ultrastructural features of erythrocyte and haemoglobin sickling. *Archives of Internal Medicine*, **133**, 545.
- WILEY, J.S. (1970) Red cell survival studies in hereditary spherocytosis. *Journal of Clinical Investigation*, **49**, 666.
- WILEY, J.S. (1972) Co-ordinated increase of sodium leak and sodium pump in hereditary spherocytosis. *British Journal of Haematology*, **22**, 529.
- WINTROBE, M.M. (1974) *Clinical Hematology*, 7th edn., p. 723. Lea & Febiger, Philadelphia.
- WITTS, L.J. (1942) Chronic leg ulcer in purpura haemorrhagica: report of two cases. *British Medical Journal*, **ii**, 309.
- WOLFORT, F.G. & KRIZEK, T.J. (1969) Skin ulceration in sickle-cell anaemia. *Plastic & Reconstructive Surgery*, **43**, 71.
- YATAGANAS, X., FESSUS, P. & GAHRTON, G. (1972) Quantification of α -chain excess in erythrocytes in β -thalassaemia by micro-interferometry. *British Journal of Haematology*, **22**, 117.
- ZAINO, E.C. & ROSSI, M.B. (1974) Ultrastructure of the erythrocytes in β -thalassaemia. *Annals of the New York Academy of Sciences*, **232**, 238.

Book Reviews

Malignant Melanoma of the Skin and Mucous Membrane. By G.W. MILTON, with chapters by V.J. MCGOVERN and MARTIN G. LEWIS (1977) Edinburgh: Churchill Livingstone. Pp. 174, 4 pages containing 32 colour plates, B/W illustrations. Price. £12.00.

In recent years several books have been written on malignant melanoma, mainly by Australians and largely based on their considerable experience with this tumour, the incidence of which in their country is probably the highest in the world. This monograph is mainly concerned with the surgical treatment of melanoma and Professor Milton gives a personal account of the methods and the therapy as carried out in his unit at the Sydney Hospital. There is an introductory first chapter by Dr McGovern on the aetiology, classification and pathology of melanoma, which reads well, is succinct, though I thought that a number of the illustrations were rather poor.

The message in this book is that melanoma should not be regarded as a death sentence and that the overall prognosis for this tumour is good. A cure can be achieved in a large proportion of cases by the wide primary excision of the tumour. What is controversial is whether the survival rate can be further improved by also carrying out a radical dissection of the regional lymph nodes. Sometimes unnecessary and occasionally mutilating surgery is performed on patients who already have wide-spread metastases that are occult. Often the regional lymph nodes when excised are unaffected. The statistics quoted for survival rates in this monograph are confusing and should have been more detailed and related to the different histogenetic types of melanoma as well as to the sites of the primary and the sex of the patients. Recent studies seem to indicate that prognosis can be related to the extent of the depth of invasion of the primary tumour into the dermis. Also, metastases to the regional lymph nodes are related to the depth of invasion of tumour cells into the reticular dermis.

This book, on the whole, is rather disappointing. Some of the topics are dealt with in a rather superficial way and there are a number of gaps that should have been covered, particularly the story about melanogens and the presence of elevated levels of cysteinyl-S-dopa in the blood and urine of patients with early primary melanomas. The best part of the book was the chapter by Martin Lewis and his associates on the immunology and immunotherapy of malignant melanoma. This contained new information and there is at the end a very comprehensive list of references.

The book will be of use to the surgeon and therefore should be in the hospital library, but I do not think it is going to be of much help to the expert nor to the dermatologist or to the pathologist.

S.S. BLEEHEN

Recent Advances in Dermatology—4. Edited by ARTHUR ROOK (1977) London: Churchill Livingstone. Pp. 395. B/W Illus. Price £12.50.

The editors and publishers of this series on advances in medicine and surgery have received considerable praise in the appropriate reviews. This latest volume on dermatology, is certainly as good as its predecessor of 1973 which it complements. Dr Rook and his colleagues have given us, as was to be expected, a first class readable and fascinating review of eleven topics, and the choice of chapters is excellent. Most of them will appeal to a much wider reading public than dermatologists. The article on tropical diseases in temperate climates for example, is most opportune and authoritative, for obvious reasons. The chapter on topical therapy is exactly what most practising dermatologists and general practitioners require, and chapters on common diseases like baldness, leg ulcers and drug reactions fit into the same category. Dr Rowell's article on ionising radiation in benign dermatoses is particularly welcome since this unnecessarily controversial subject is usually given scant attention in the British literature, even in the best text books. Many dermatologists neglect this useful therapeutic measure which is not always easily available for their patients. Radiotherapy instruction given to dermatologists in training, often lays too great

a stress on malignant disease to the exclusion of much else. The chapter on sexually transmitted diseases is of great practical use and its medical political comments, although well known to dermatologists, may surprise and interest other readers. This book can be very strongly recommended indeed to dermatologists, general practitioners and many others besides. If some should demur at its cost, it should be pointed out that the lists of references, given at the end of each chapter, are alone well worth the price of the book.

O.L.S. SCOTT

News and Notices

Dermatopathology Course

A Dermatopathology Foundation Course in Gross and Microscopic Pathology of the Skin will be held under the auspices of Tufts University, Department of Continuing Education, from 12 to 16 June 1978 at Howard Johnson Motor Lodge, Boston, MA, U.S.A. Speakers include Dr Milton R. Okum, Dr Leon M. Edelstein and Dr Walter F. Lever.

This is a five-day intensive course with simultaneous projection of Gross and Microscopic colour 35 mm slides (40 hour credit, AMA category 1a).

For details and applications contact: Dr R.P. Patel, Ph.D., Dermatopathology Foundation, PO Box 377, Canton, MA. 02021, U.S.A.

Irish Association of Dermatologists

The Spring Meeting of The Irish Association of Dermatologists will be held in Cork on 28–29 April 1978. There will be short symposia on Contact Dermatitis, Light Sensitivity, Vasculitis and Dermatological Education, and a clinical meeting.

Visitors are welcome, but numbers restricted. Further details may be obtained from Dr R.S. Matthews, Consultant Dermatologist, Route Hospital, Ballymoney, Co. Antrim.

Paediatric Dermatology Course

A course in Paediatric Dermatology will be held at Arcachon, near Bordeaux, France, on 28 March–1 April 1978. Subjects to be discussed include acute and chronic erythematous eruptions in infancy, erythematous-squamous eruptions in infancy, bullous eruptions in infancy, the intestine and bullous eruptions. There will be a clinical meeting in Bordeaux.

Enquiries to: Secrétariat du service de Dermatologie, Hopital des Enfants, 168 Cours de l'Argonne, 33077 Bordeaux, France.

APPOINTMENTS

Sunderland and South Tyneside Health Area

Dr B. Gordon has been appointed Dermatologist to Sunderland Royal Infirmary. He qualified M.B. B.S., Newcastle-upon-Tyne, 1943 and served in the R.A.M.C. 1944–1947. He was then in general practice and at the same time acted as Clinical Assistant in Dermatology in Sunderland from 1952 to 1977. His publications include 'The Use of Topical Proteolytic Enzymes in the Treatment of Post-thrombotic Leg Ulcers'.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometer(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	¹³¹ I
hour(s)	h	milliequivalent	mmol		

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Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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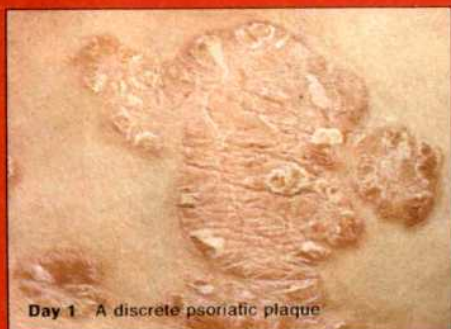
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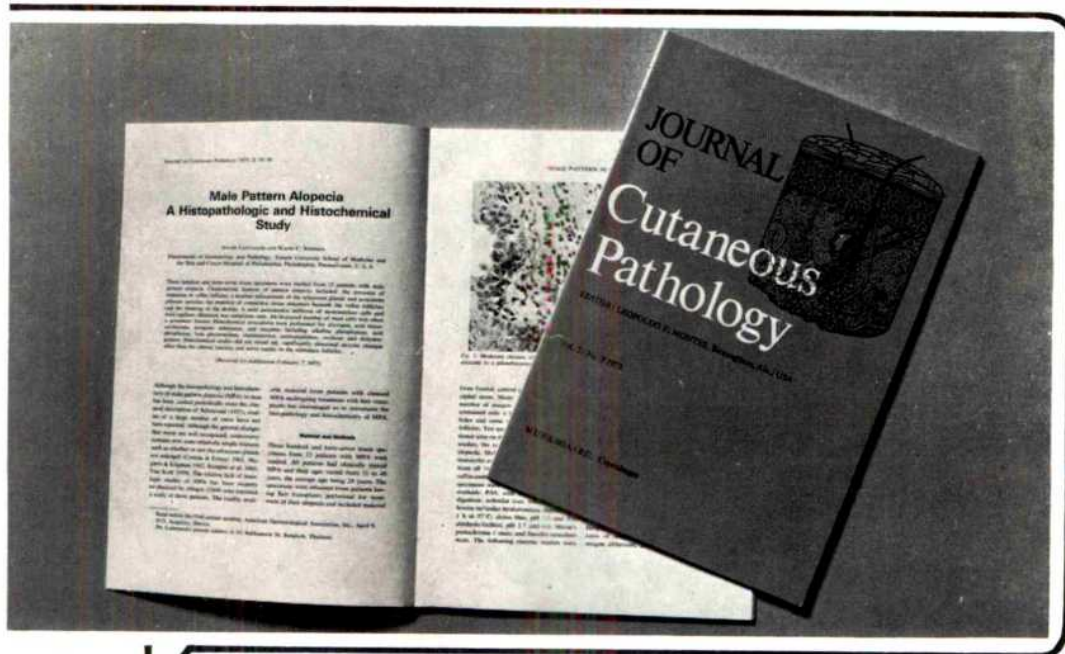
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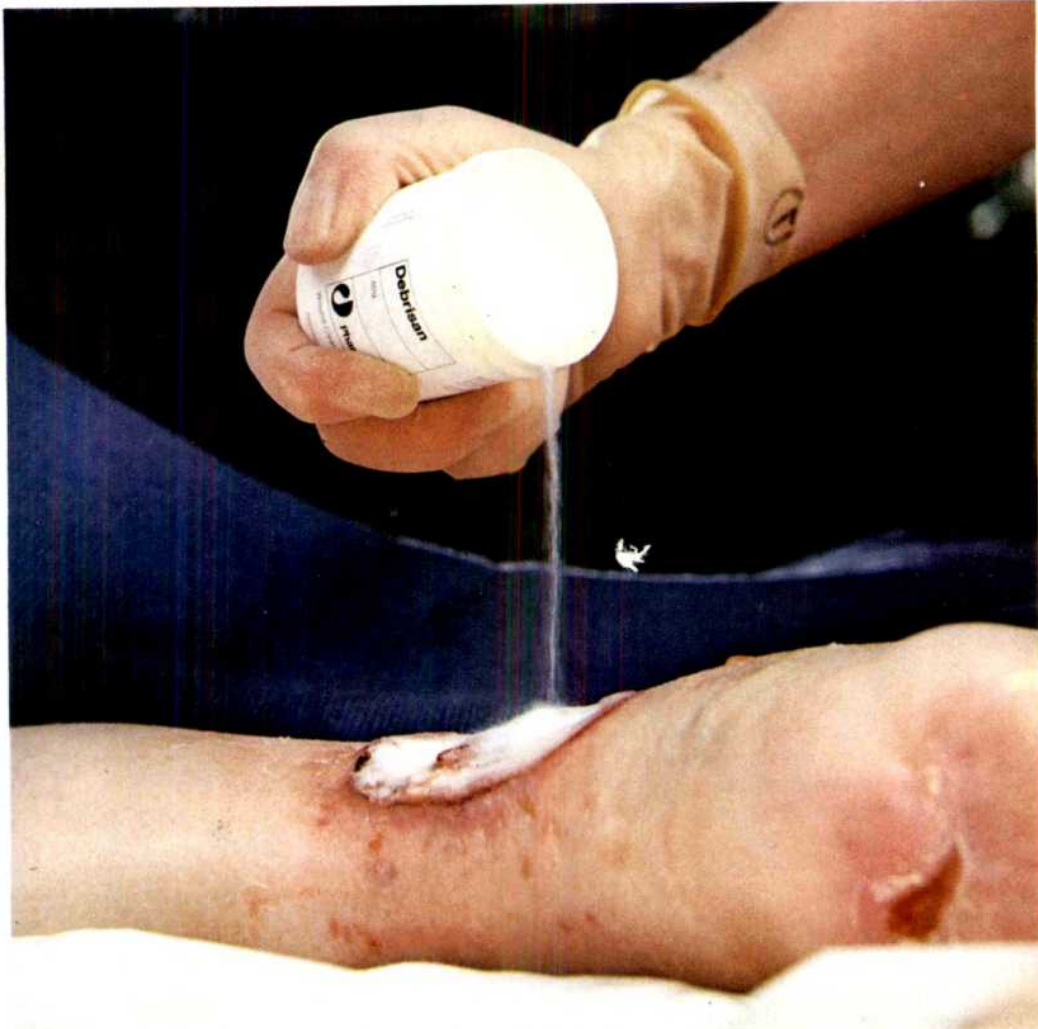


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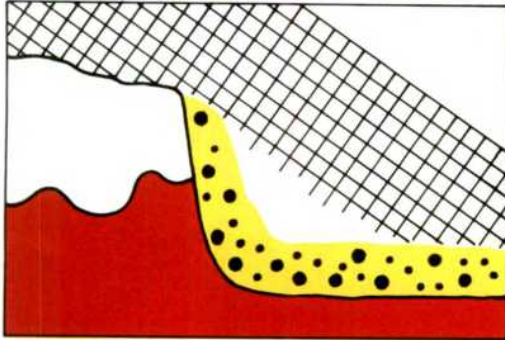
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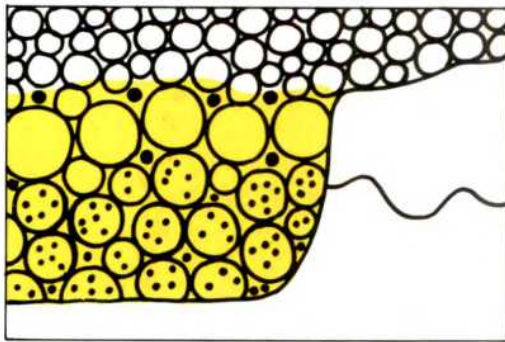
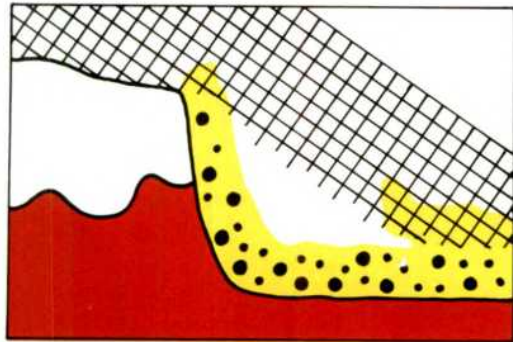
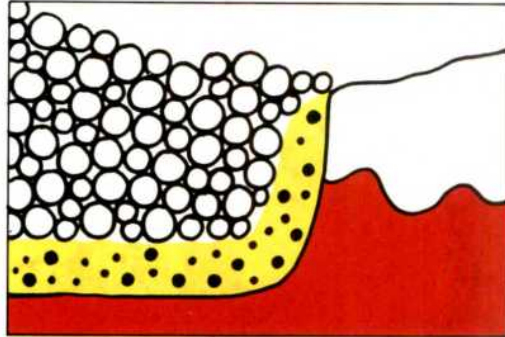
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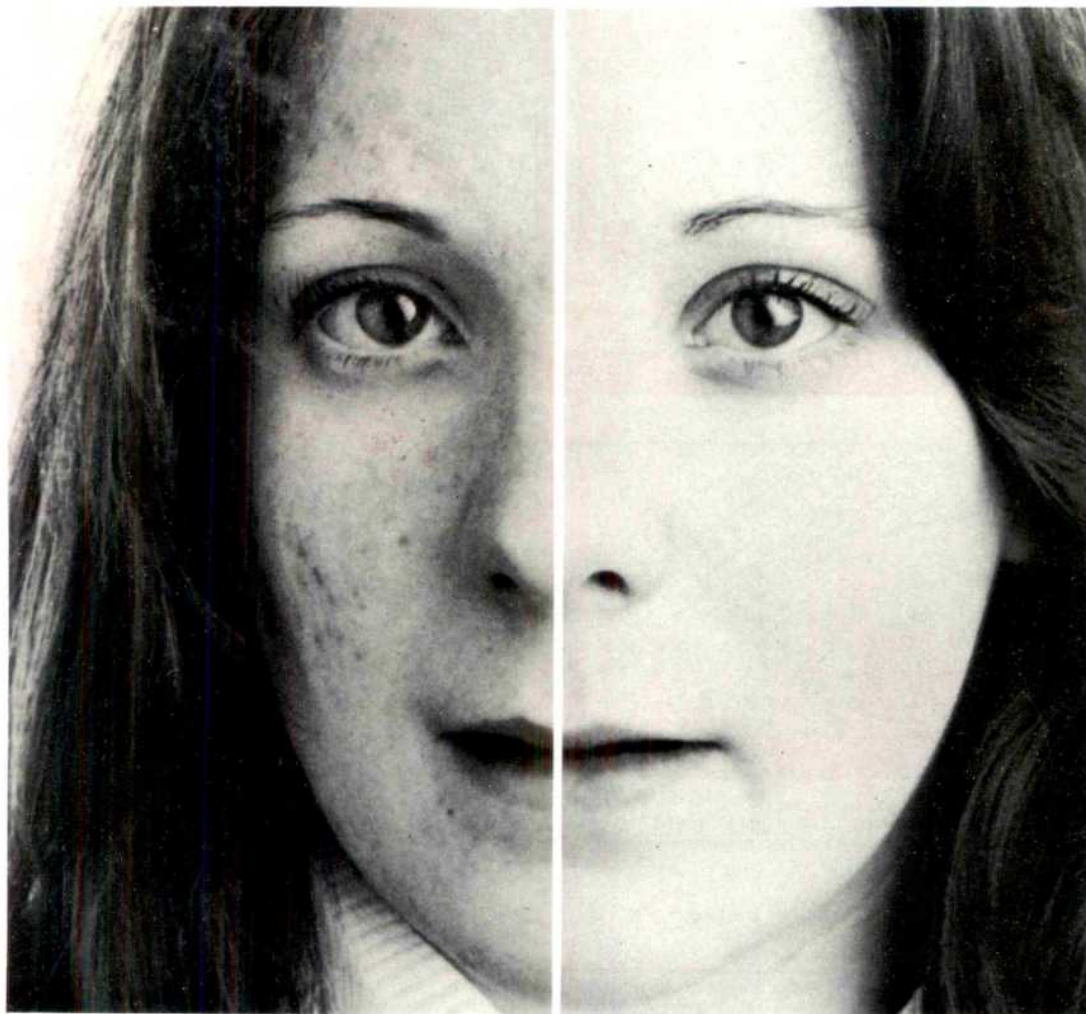
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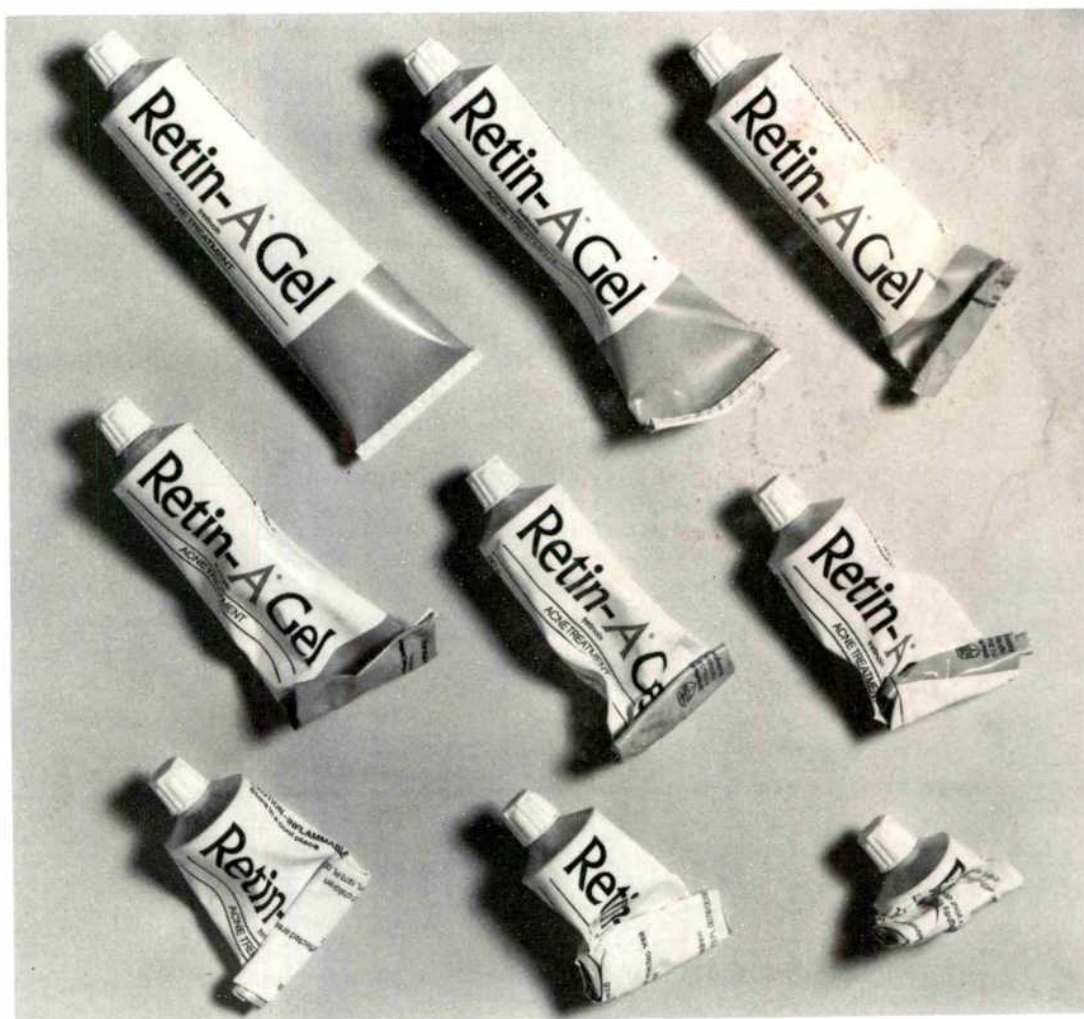
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1. Simpson, J. R. et al., (1973) Current Medical Research and Opinion, 1:485. 2. Mills, O. H. et al., (1972) Archives of Dermatology, 106: 200. 3. Leyden, J. J. et al., (1974) Southern Medical Journal 67: 20. 4. Mandy, S. H. (1974) Cutis 14: 855-857.

Symposia of the Zoological Society of London Number 39

Comparative Biology of Skin

edited by
R. I. C. Spearman

November 1977
xviii + 428pp., £14.80/\$29.00
0.12.613339.5

The skin of animals provides the most important interface between living cells and a diverse and often hostile environment. It not only acts as a barrier but may also occasionally be involved in skeletal function or may have to withstand high internal pressures of body fluids. The variations in skin structure and function which have developed to cope with these problems, in varied groups of animals, are the subject of this symposium volume. Important aspects of epidermal structure and function and melanogenesis are treated in detail by authorities in their fields. Coverage of both vertebrates and invertebrates is especially useful and the broad comparative aspects of such topics as fibrous proteins, cuticles and mucous secretion is unique.

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HISTOPATHOLOGY OF THE SKIN

Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

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Cellular fine structure in the invasive nodules of different histogenetic types of malignant melanoma

J.A.A.HUNTER,* S.ZAYNOUN,* W.D.PATERSON,*
S.S.BLEEHEEN,† RONA MACKIE‡ AND A.J.COCHRAN‡

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SUMMARY

Ultrastructural studies were carried out on the invasive nodule of forty malignant melanomas. The findings support the concept that the fine structure of lentigo maligna melanoma is often characteristic, and differs from that of superficial spreading and nodular melanoma. The melanosomes in lentigo maligna melanoma are usually ellipsoidal and resemble those of normal melanocytes, whereas the melanosomes in superficial spreading and nodular melanoma are most often spheroidal and abnormal in appearance. Superficial spreading and nodular melanomas cannot be distinguished reliably by their ultrastructure.

Melanosomal appearances could not be related to the presence of a pre-existing naevus or the depth of invasion of the tumour nodule.

The clinico-pathological classification of malignant melanoma of the skin remains imperfect, but the classification suggested by Clark *et al.* (1969) and McGovern (1970) is gradually gaining acceptance (McGovern *et al.*, 1973). This classification recognizes three major types of invasive malignant melanoma which differ in their mode of onset, course and prognosis. They have distinctive clinical and histological appearances (McGovern *et al.*, 1973) and two are preceded by an *in situ* phase of intra-epidermal proliferation of malignant melanocytes (Fig. 1).

Invasive malignant melanoma with adjacent intra-epidermal change of lentigo maligna type (Fig. 1c) is preceded by the *in situ* changes of lentigo maligna (Hutchinson's freckle, melanose circonscrite précancéreuse of Dubreuilh, mélanosis circumscripta preblastomatosa) (Fig. 1a). The pre-invasive phase exists on average for 10–15 years though in the individual case may be shorter or longer. Invasive malignant melanoma with an adjacent intra-epidermal change of superficial spreading type (Fig. 1d) is preceded by superficial spreading melanoma *in situ* (pagetoid melanoma, premalignant melanosis) (Fig. 1b). The pre-invasive phase may only exist for a few months. Invasive malignant melanoma without adjacent intra-epidermal change (Fig. 1e) apparently appears *de novo*—with no

Correspondence: Dr J.A.A.Hunter, Department of Dermatology, University of Edinburgh, Edinburgh.

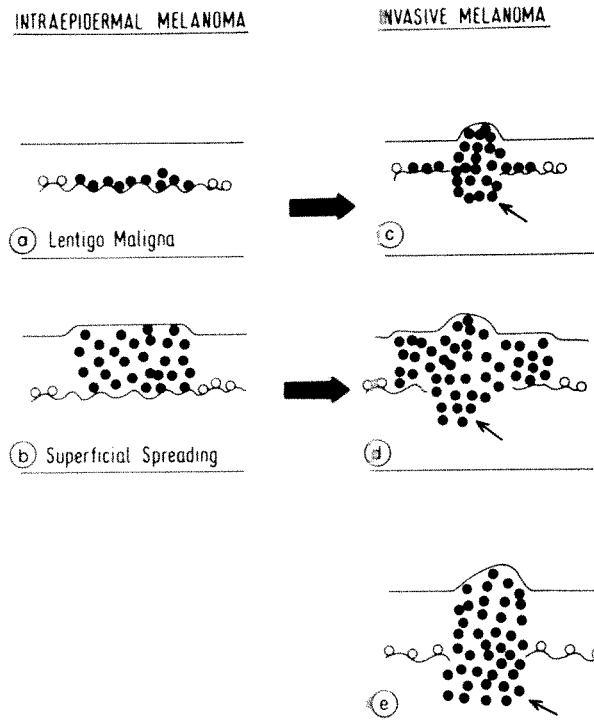


FIGURE 1. Histogenetic types of malignant melanoma. The small arrows point to part of tumour examined. (○) Normal melanocyte; (●) malignant melanocyte.

clinically overt antecedent pigmentation and no histologically detectable melanocytic anomaly in the adjacent epidermis.

A fourth subgroup is also permitted in the classification: invasive malignant melanoma with adjacent intra-epidermal change of unclassifiable type. This covers the cases where changes in the surrounding epidermis are histologically anomalous, and should represent only a very small number of cases where both clinical and histological data are available for inspection.

Although there is some agreement on the histology of the different types of melanoma, it is not clear whether these variations show distinguishing ultrastructural features.

Studies of the fine structure of melanosomes in melanoma were initiated by Mishima (1960, 1967). On the basis of clinical and pathological features he suggested that melanomas arise either by malignant transformation of normal melanocytes (melanocytic malignant melanoma) or by transformation of junctional naevus cells (naevocytic malignant melanoma). He supported this hypothesis with evidence that melanocytic and naevocytic malignant melanomas differed in the fine structure of their melanosomes. Although Mishima's hypothesis has been questioned, his ideas have stimulated extensive studies of melanosome polymorphism in melanomas of different histogenetic types. There has been little agreement between the findings of the various studies. Much of the published material, however, is based on small series which are likely to give rise to sampling errors and, in previous studies, the histogenetic type of tumour was usually known to the microscopist, raising the possibility of observer bias in interpretation of the micrographs.

The present study was undertaken to determine whether the histogenetic types of melanoma have specific ultrastructural characteristics. One group (RM and AC) classified the melanomas by histo-

genetic category and the other (JH, SZ, WP and SB) was responsible for the electron microscopy without prior knowledge of the histology. After both groups had recorded their findings the code was broken and the two sets of observations correlated. The preliminary findings have already been presented (Hunter *et al.*, 1976).

MATERIALS AND METHODS

Histogenetic typing

Forty excised melanomas (37 primary tumours and 3 secondary deposits) were studied. Case summaries, clinical photographs and appropriate representative sections stained with haematoxylin and eosin and Masson's trichrome stain were collected (WP) and sent to Glasgow (RM and AC) and the histogenetic type and depth of invasion of each tumour were assessed by the criteria of McGovern *et al.* (1973).

Antecedent pigmented lesions

The existence of an antecedent pigmented lesion was assessed on historical evidence. Three descriptions only were accepted as indicating a probable preceding naevus (as opposed to the *in situ* phase of melanoma growth), e.g. pigmented lesion 'present all life', 'present since a child' or 'present as long as I can remember'. Excluded as naevi were pre-existing pigmented lesions which could be traced back for up to 20 years. It was felt that some true pre-existing naevi might be excluded using this method of assessment, but those accepted seemed reasonably certain instances. Histological evidence of naevi was not sought because it was felt that reliable assessment could be made only by examining serial sections of the whole lesion. This was not possible as parts of the tumour were prepared for the electron microscopy described in this study and some further fragments were submitted for other studies involving cell culture.

Electron microscopy

Standard techniques were employed. Tissue was taken from the invasive nodule (arrowed in Fig. 1) of freshly excised melanomas and fixed for 3–5 h at 25°C in half strength Karnovsky's formaldehyde-glutaraldehyde fixative (Karnovsky, 1965) and post fixed in osmium tetroxide. After block staining with uranyl acetate, the cubes were dehydrated and embedded in araldite in a routine manner.

Thick sections of the block face were cut and stained. The technician then selected an area of the block for ultra thin sectioning. Thin sections were cut, contrasted with lead citrate and uranyl acetate and viewed on an A.E.I. Corinth 275 electron microscope at 50 kV.

Electron microscopy was carried out in Edinburgh by J.H. and S.Z. and, as neither worker was involved in the thin sectioning, they were unaware of the tumour type. A minimum of 30 random photographs of each specimen, selected from at least 2 blocks, were taken usually at a machine magnification of $\times 6000$. Electron micrographs were analysed independently in Edinburgh (J.H.) and Sheffield (S.B.) using a standardized proforma (Table 1), the histogenetic type of the tumour still being unknown to both workers.

Particular attention was directed to the morphology of melanosomes which were categorized as ellipsoidal or spheroidal and further subdivided into normal, abortive, granular and lamellar types according to the terminology of Clark *et al.* (1972). A further type of melanosome, termed vacuolar (see below), was identified and considered because of its frequent occurrence. The frequency of each type of melanosome was scored (Table 1).

Normal melanosomes. Normal melanosomes in melanoma cells were observed as described and

TABLE 1. Melanoma proforma

MELANOMA STUDY NO:				
(Circle relevant finding)	+ + + = Nearly all + + = Frequent + = Occasional but rare - = None			
TUMOUR CELLS				
Nucleus:	Normal	Convolutd		
Nucleolus:	Normal	Abnormal—Compact —Dispersed		
Golgi:	Normal	Prominent		
Mitochondria:	Normal	Prominent		
R.E.R.:	Normal	Prominent		
Free ribosomes:	Normal	Prominent		
MELANOSOMES				
Size:	nm	nm		
Shape:	Ellipsoidal	Spheroidal		
	+ + + + + -	+ + + + + -		
Character:	Normal:	+ + + + + -		
	Abortive:	+ + + + + -		
	Vacuolar:	+ + + + + -		
	Granular:	+ + + + + -		
	Lamellar:	+ + + + + -		
Amount of pigment:	Normal	Much		Little
Distribution of pigment:	Normal	Abnormal		
Autophagosomal profiles:	None	Few		
DIFFERENTIATION OF TUMOUR CELLS				
Normal (A)	Moderate (B)		Poor (C)	None (D)
CLARK TYPING	I	II	III	IV

illustrated in standard texts (e.g. Zelickson, 1967) although they may be larger or smaller than those found in normal melanocytes. They are ellipsoidal and, before melanisation is complete, reveal an internal structure composed of individual melanofilaments with distinctive periodicity (80–100 Å) and a tendency to form cross striations.

Abortive melanosomes. These were spheroidal organelles containing one or more melanofilaments having the distinctive periodicity of the filaments of a normal melanosome. Filaments are, however, not present in large numbers in parallel array, and show no tendency to form 'cross-linkages'. Striated appearances are therefore rare in this type of melanosome.

Granular melanosomes. These are spheroidal organelles, limited by a unit membrane, and containing an evenly dispersed component of small granular structure (approximately 6 nm in diameter).

Lamellar melanosomes. These are spheroidal organelles characterized by an internal array of unit membranes forming either a concentric whorl or parallel patterns. They may also contain clusters of vesicles, similar to a compound lysosome.

Vacuolar melanosomes. These are empty spheroidal organelles, limited by a unit membrane. Their melanosomal nature could only be identified with certainty by their dopa positivity (Hunter, Paterson & Fairley, 1978).

Vacuolar melanosomes never contain melanin but pigment, which may be regular and organized, or totally haphazard, occurs in all other types of melanosomes.

Considering the fine structural features the observers attempted to grade the degree of subcellular differentiation within the tumour. If the tumour cells resembled normal melanocytes, this was regarded as 'normal differentiation' (A). 'No differentiation' (D) described tumours with cells containing no recognizable melanosomes. Moderate differentiation (B) described tumours containing cells with both normal and abnormal melanosomes. Poor differentiation (C) described tumours with cells containing melanosomes which were almost entirely abnormal. Broad categories were chosen in view of the subjective nature of this opinion (Table 1).

Finally, an attempt was made to classify the tumour by its predominant cell, again using the terminology of Clark *et al.* (1972).

Type I cell: melanoma cell containing mostly normal melanosomes.

Type II cell: melanoma cell containing mainly abortive melanosomes.

Type III cell: melanoma cell containing mainly vacuolar, granular and lamellar melanosomes.

Type IV cell: an unidentified cell often with few or no melanosomes. The nucleus is frequently convoluted and the cytoplasm crowded with mitochondria and free ribosomes.

RESULTS

Histogenetic typing and depth of invasion

Study of case summaries, clinical photographs and light histological material allowed classification of the tumours according to their histogenetic type and depth of invasion (Table 2).

TABLE 2. Number of specimens categorized according to histogenetic type and depth of invasion

Histogenetic type	Total examined	Level II	Depth of invasion		
			Level III	Level IV	Level V
Lentigo maligna melanoma	14	4	4	4	2
Superficial spreading melanoma	14*	2	5	3	3
Nodular melanoma	9	0	2	5	2
Secondary cutaneous deposit	3		Not assessed		

* Depth of invasion could not be assessed in one specimen.

Electron microscopy

General cellular characteristics. There were only minor variations in the general cellular architecture of tumours of different histogenetic types. The nuclei of all types of tumour cells were frequently bizarre and convoluted (Fig. 2). The nucleoli were often abnormally prominent and the nucleolonema was either a compact mass or was dispersed as strands (Fig. 2). Mitochondria and free ribosomes were plentiful and the Golgi apparatus prominent in some. No particular appearances were characteristic of any histogenetic type.

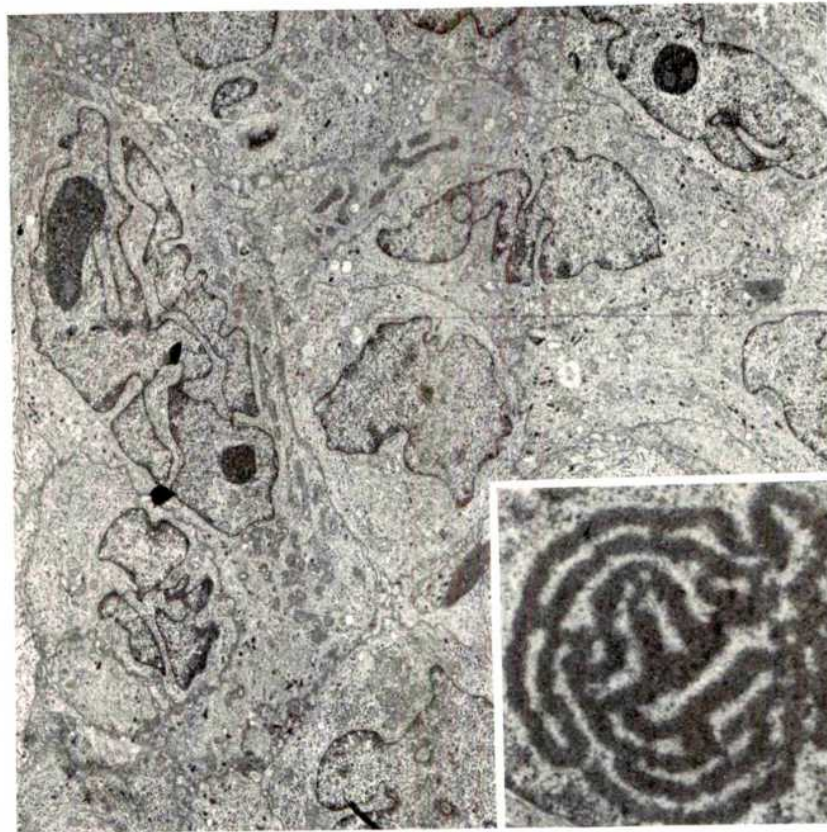


FIGURE 2. Convoluted nuclei and prominent nucleoli in tumour cells ($\times 4000$). Inset: nucleolus of tumour cell is composed of concentric strands of nucleolonema ($\times 16,500$).

Tumour cells in lentigo maligna melanoma, however, often exhibited numerous dendritic processes, a feature seldom seen in the other types.

In general most cells in a given tumour were similar, and in only one specimen was there much difference between the two blocks examined. However, it was obvious that in all tumours there were occasional cells which were different from the main type. For instance most cells of a tumour might contain numerous granular melanosomes and a prominent Golgi, but amongst these were occasional cells with no or very few melanosomes, but numerous mitochondria and free ribosomes. It was therefore important to pick out the predominant cell type.

Some tumour cells contained obvious homogeneous lipid inclusions whereas others contained vacuoles demarcated by a layer of lipid. Occasionally large ($0.8\text{--}1.4\text{ }\mu\text{m}$) empty vacuoles, with no limiting membrane, were seen (Fig. 9) and it seemed likely that they were a result of lipid extraction during processing. These vacuoles were noted with equal frequency in all histogenetic types and were quite different from vacuolar melanosomes, which were smaller ($150\text{--}400\text{ nm}$) and demarcated by a unit membrane.

Langerhans cells in the epidermis overlying the tumour nodules appeared normal, though on a few occasions they contained phagocytosed melanin.

Macrophages containing pigment (melanophages) and other mononuclear inflammatory cells were

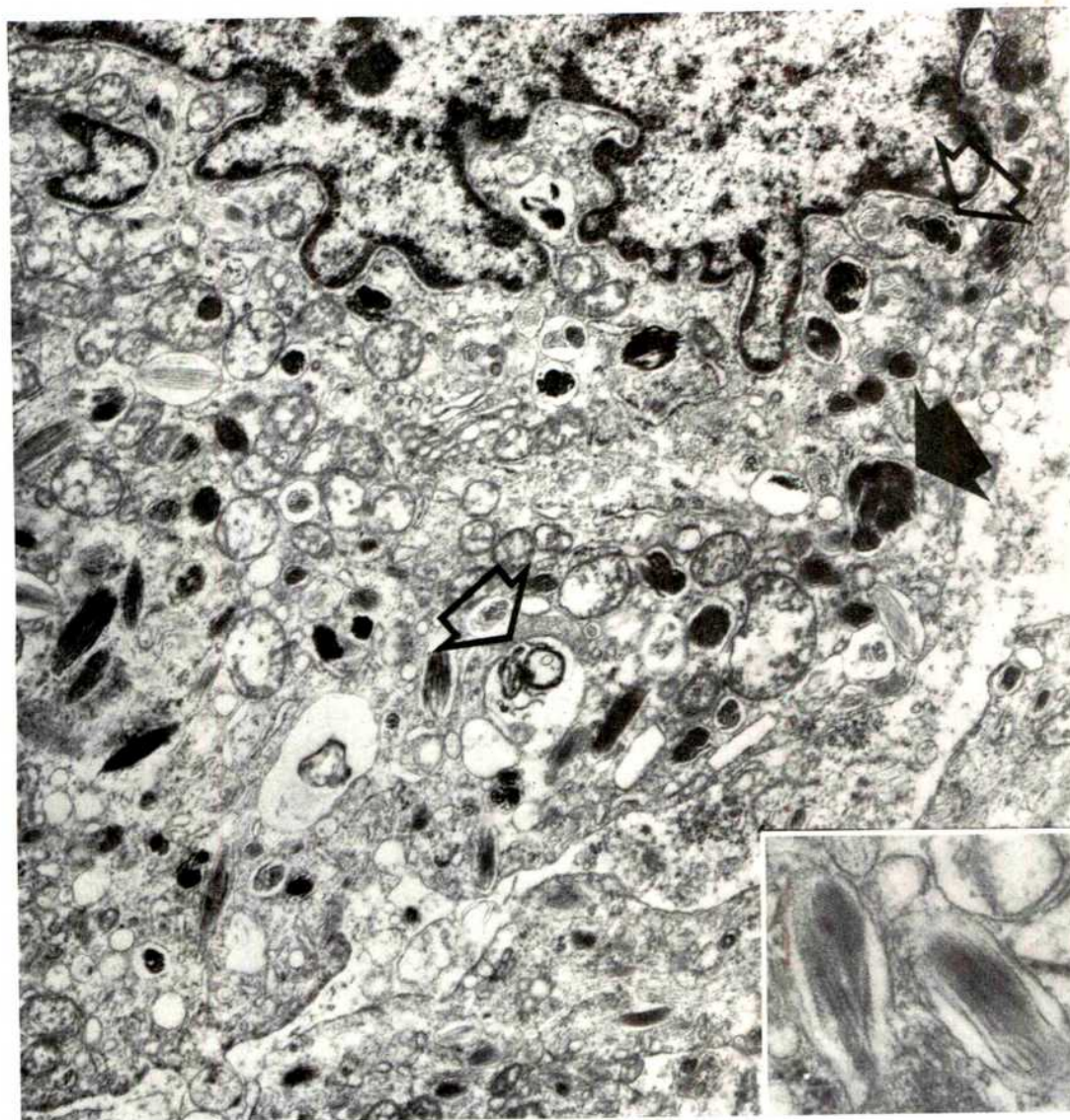


FIGURE 3. Type I cell with numerous relatively normal ellipsoidal melanosomes. Occasional abnormal melanosomes (open arrows) are seen. Arrow points to autophagic vacuole ($\times 16,000$). Inset: Ellipsoidal melanosomes in Type I cell. Filaments show cross striation with periodicity of 80 Å ($\times 57,000$).

seen amongst the tumour cells in all specimens. They were not studied in detail, but their presence or absence did not seem typical of any histogenetic type. Sometimes it was not easy to differentiate between melanophages and tumour cells showing marked autophagocytosis of melanin. However, in the latter, some melanosomes disposed individually (rather than in complexes) were apparent (Fig. 10).

Melanosomal morphology. Particular attention was paid to the type of melanosome seen in the tumour

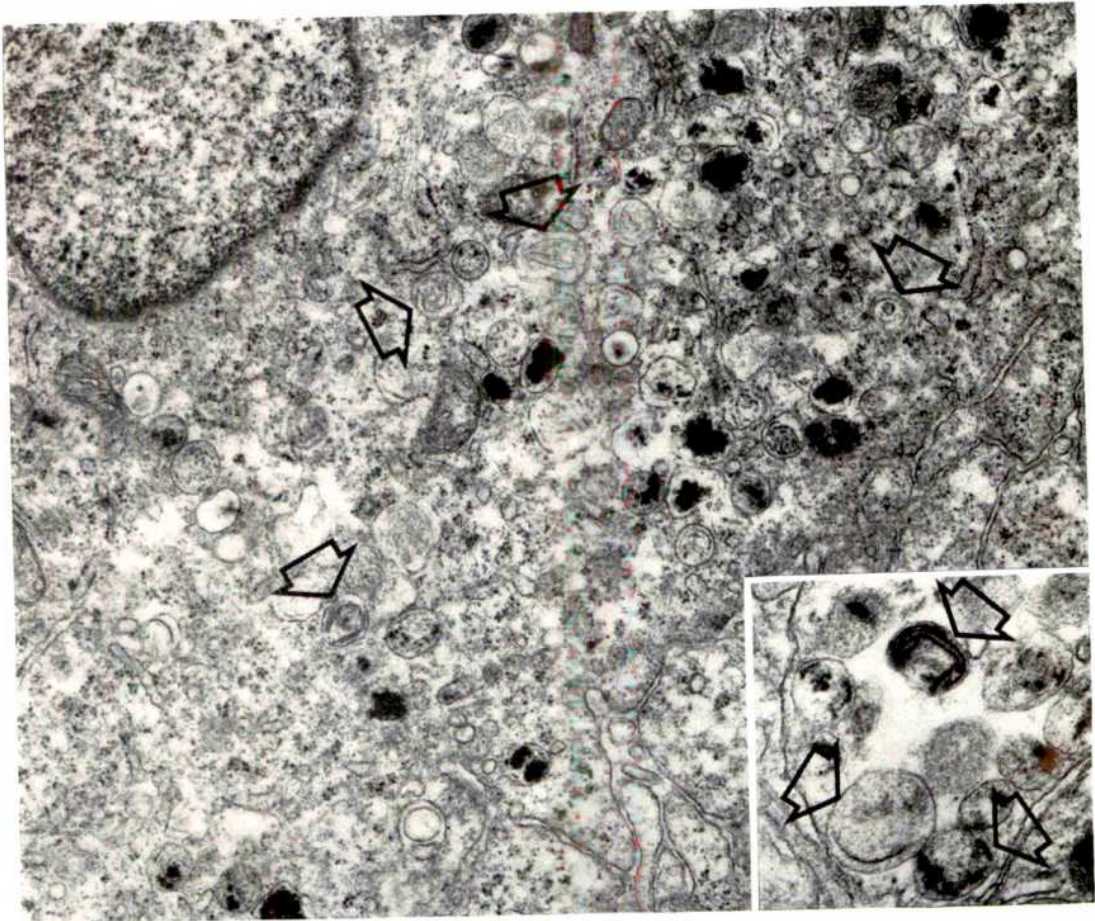


FIGURE 4. Type II cell containing numerous abortive melanosomes (open arrows). Filaments in these are orientated in a haphazard manner and there is no cross linking. Irregular pigment deposition is seen in many melanosomes ($\times 21,000$). Inset: Abortive melanosomes are arrowed ($\times 32,000$).

cells. Figs. 3–8 illustrate the characteristic appearance of each type. Once again no particular appearance was unique in a single tumour, but it was usually easy to determine the most common melanosomal forms in each tumour, and their frequency was noted on the proforma.

The degree of autophagocytosis of melanosomes (Fig. 10) within tumour cells was also recorded on the proforma.

Eighty proformas were analysed (one proforma for each specimen by each observer) and there was, in all but a few instances, good agreement between the two workers. Tables 3, 4 and 5 summarize the findings in all the specimens examined.

The ultrastructural appearances of lentigo maligna is, in the majority of cases, quite characteristic. The cells are frequently dendritic and it is the only type of melanoma in which large numbers of ellipsoidal and normal looking melanosomes ($150\text{--}300\text{ nm} \times 400\text{--}500\text{ nm}$) are often seen. Granular melanosomes are seen only rarely. In general the cells in lentigo maligna melanoma exhibit a high degree of differentiation, which approaches the picture seen in the normal melanocyte.

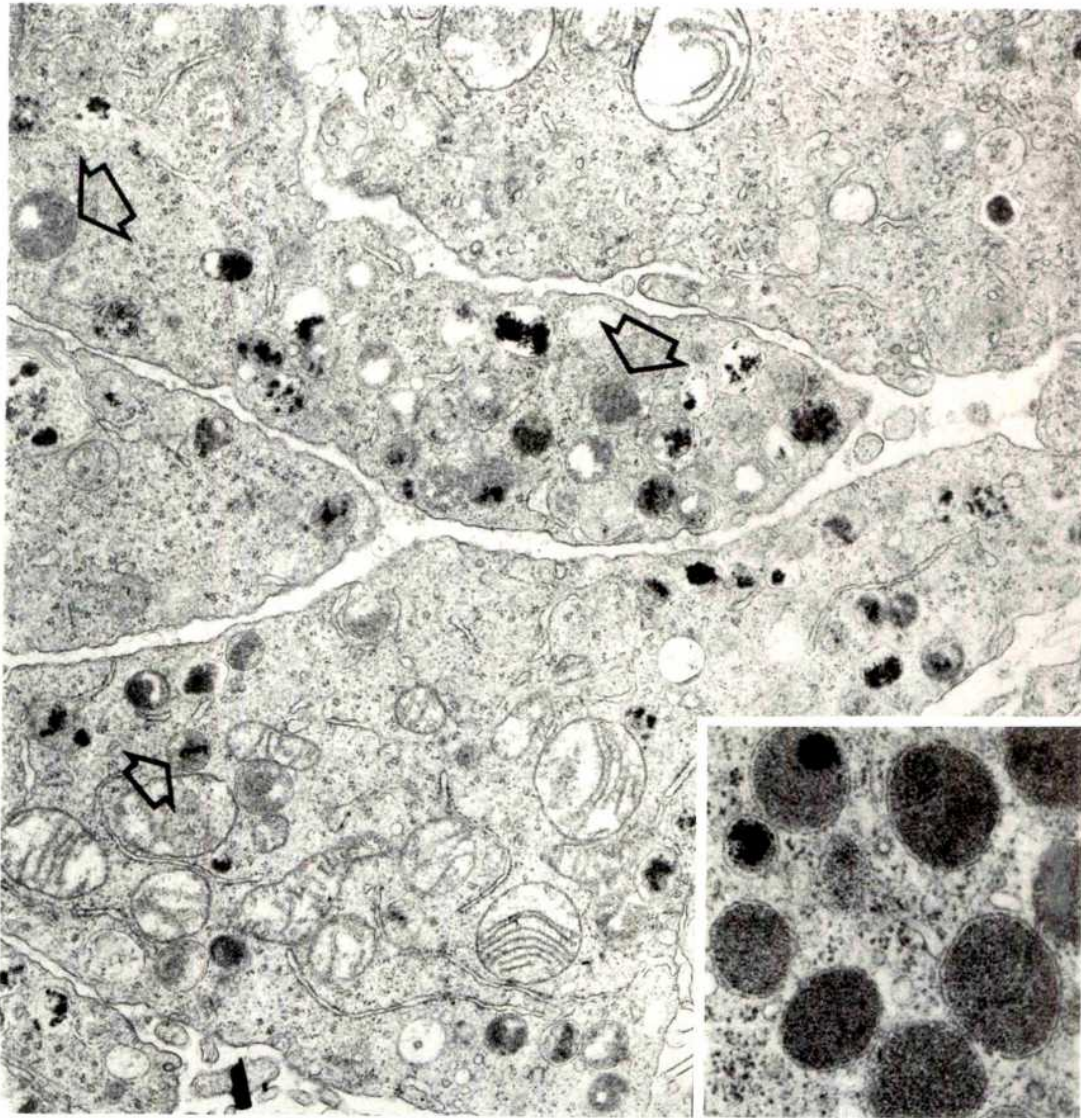


FIGURE 5. Type III cell containing numerous granular melanosomes (arrows) as well as occasional abortive and lamellar organelles. Random pigment deposition is seen in many melanosomes ($\times 16,000$). Inset: granular melanosomes. There is pigment deposition in some ($\times 37,000$).

The tumour cells in all the other types of melanoma are similar. They usually contain numerous spheroidal melanosomes (150–500 nm in diameter) with only occasional ellipsoidal profiles. Abortive and granular melanosomes are common in all, though lamellar organelles are seen less frequently. Vacuolar melanosomes appear to be more common in superficial spreading melanomas. The tumour cells in superficial spreading, nodular and secondary melanomas are therefore rather poorly differentiated, and often bear little resemblance to normal melanocytes.

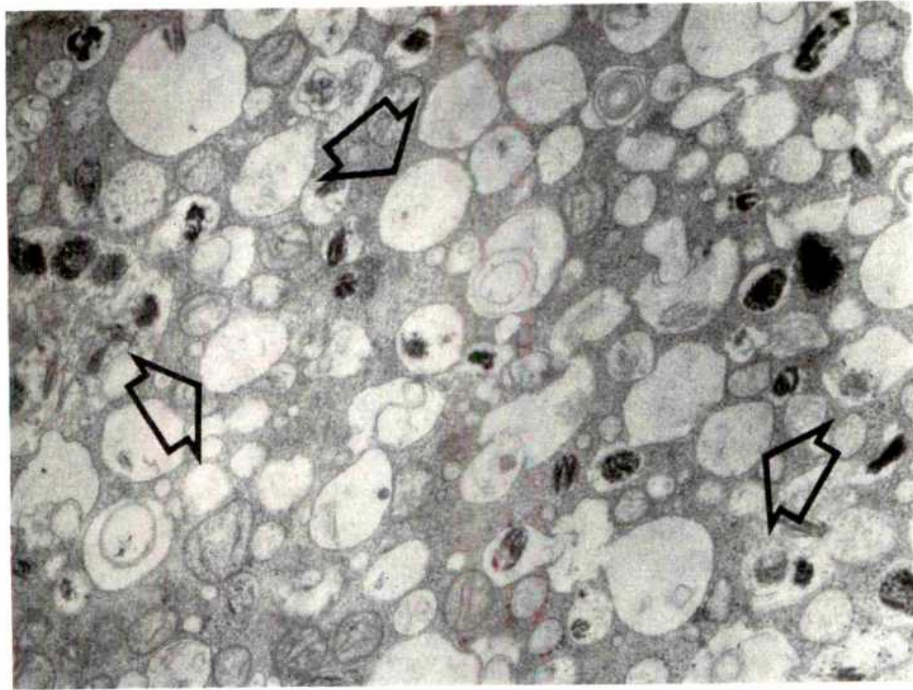


FIGURE 6. Vacuolar melanosomes (arrowed). Empty vacuoles (300–600 nm in diameter) lined by a limiting membrane and surrounded by other abnormal spheroidal melanosomes ($\times 19,000$).

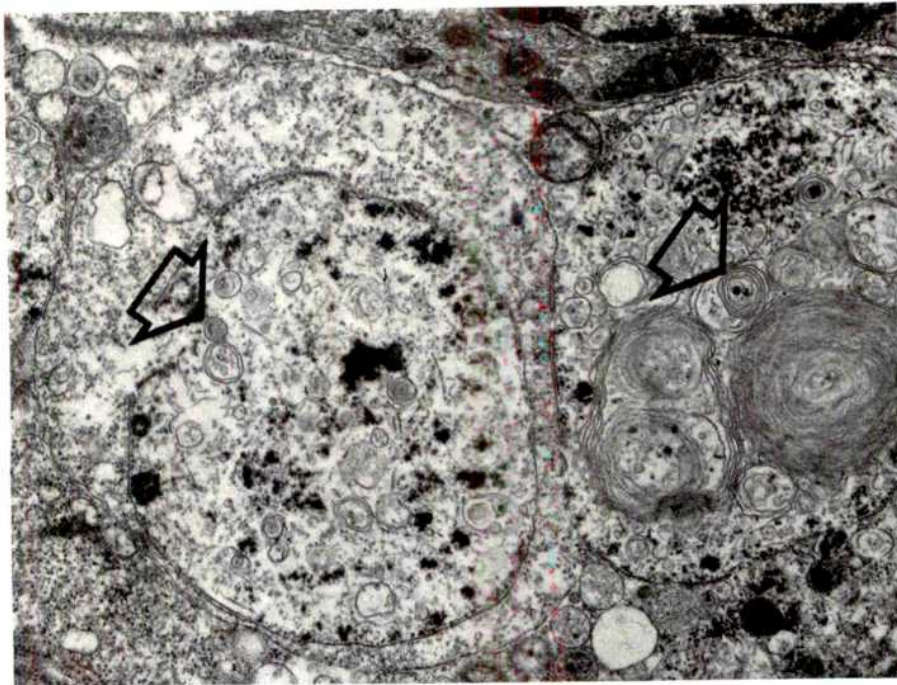


FIGURE 7. Lamellar melanosomes (arrowed). The organelle on the right lies within a large autophagic vacuole ($\times 18,500$).



FIGURE 8. Type IV cells. Free ribosomes are prominent, but very few melanosomal structures are seen ($\times 11,500$).

Autophagocytosis is seen frequently in lentigo maligna melanoma and superficial spreading melanoma, but is less common in nodular melanomas.

Melanomas apparently arising in association with pre-existing naevi were not characterized by the predominance of any particular type of melanosome. They exhibited the complete spectrum of melanosomal profiles. Likewise the depth of tumour invasion could not be correlated with any particular melanosomal picture: i.e. deeper invading tumours did not necessarily contain more undifferentiated cells.

DISCUSSION

Findings in this study

There is a high percentage (37.8% of primary tumours) of lentigo maligna melanomas in this study when compared with the series of Clark *et al.* (1969) (13.9%), McGovern (1970) (10%) and Kopf *et al.* (1977) (4.9%). It is possible that this reflects the interests of the different departments involved

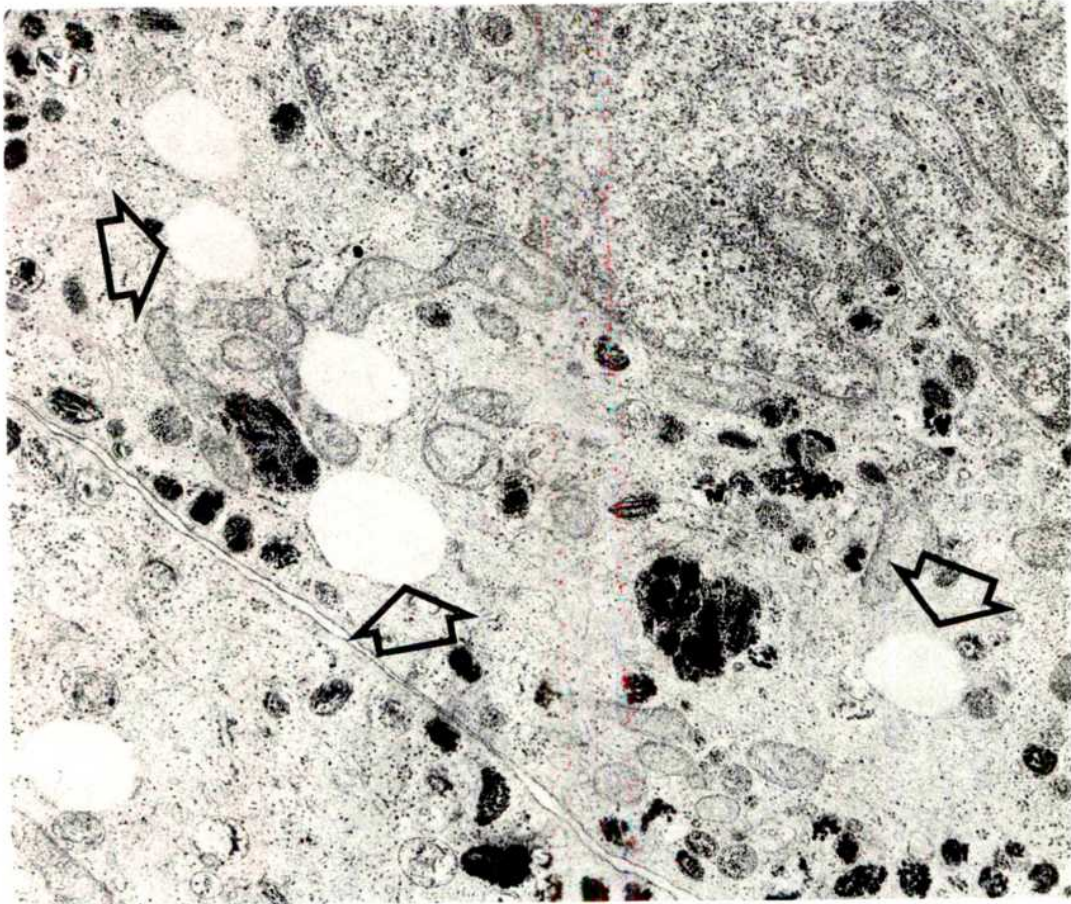


FIGURE 9. Arrows point to vacuoles (up to $0.8 \mu\text{m}$ in diameter) in tumour cells. There is no limiting membrane to the vacuoles, which have probably developed as a result of lipid extraction in the processing procedure ($\times 24,000$).

in this study. Many specimens came from the plastic surgery and dermatology units where old people (average age 66 years in our series) tend to be referred with this type of tumour.

Our results indicate that the ultrastructure of lentigo maligna melanoma is fairly characteristic. This has been confirmed in a separate ultrastructural study carried out in Sheffield on multiple biopsies taken from eight patients with lentigo maligna melanoma (Bleehen, Hartley & Senior, 1976). The evidence suggests that this is a well differentiated tumour composed of cells showing malignant features (in particular large convoluted nuclei and abnormal nucleoli) but with relatively normal melanogenesis.

In most cases this picture is in direct contrast to that seen in superficial spreading, nodular and secondary melanomas. Here 'anarchy displaces order' (Cesarini, 1971) and melanogenesis not only occurs in abnormal spheroidal organelles, but becomes haphazard and disorganized. There are only minimal differences in the ultrastructure of superficial spreading, nodular and secondary melanomas. The incidence of abortive, granular and lamellar organelles seems similar in each, though vacuolar

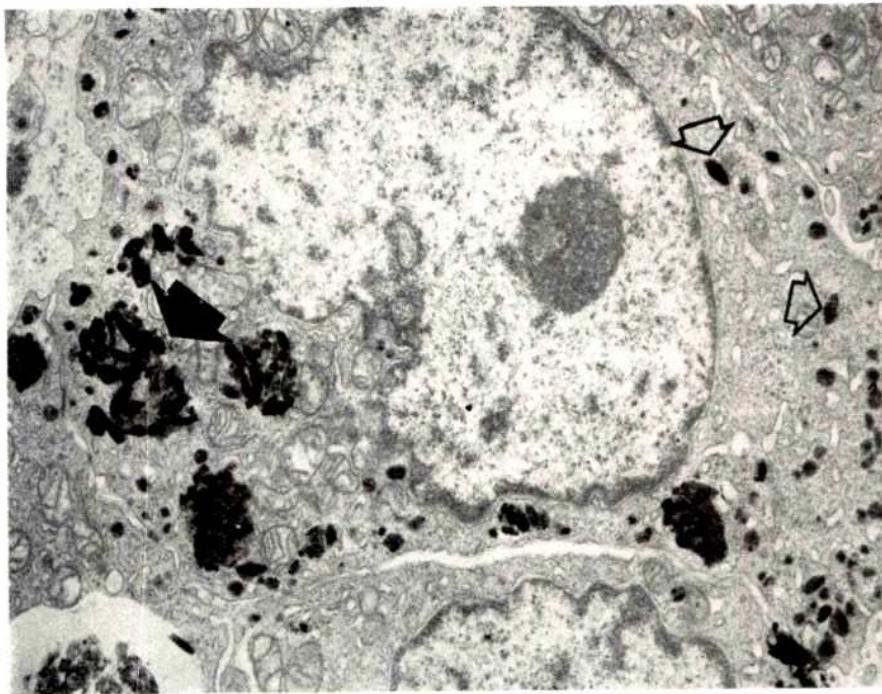


FIGURE 10. Autophagocytosis in tumour cell (arrow). Note melanosomes disposed individually (open arrows) in same cell ($\times 10,000$).

TABLE 3. Melanosomal characteristics in lentigo maligna melanoma

Series no.	Level of invasion	Predominant cell type	Grade of differentiation	Melanosomes							Autophagocytosis
				Spheroidal	Ellipsoidal	Normal	Abortive	Vacuolar	Granular	Lamellar	
Lentigo maligna melanoma											
8	2	II/III(IV)†	B	+	++	++	++	-	+	-	+
9	3	IV(III)	C	+++	+	-	+	+	+	+	-
11	4	II/III	B	+	++	++	++	+	+	-	+
12	3	I/II	B	+	++	++	++	+	+	+	++
13	2	III(II)	C	+++	-	-	++	+	++	+	+
22*	2	II(III)(IV)	B	++	++	++	++	+	+	+	++
24	4	III(II)	C	+++	-	-	++	+	++	+	+
28*	5	I/II	B	+	++	++	+	+	+	-	++
29	4	III(IV)	C	++	++	+	++	+	++	-	+
30	3	II(III)(IV)	B	+	+++	++	+	-	+	+	++
34	2	I	A	+	+++	+++	+	-	-	-	++
40	4	II	B	++	++	++	++	+	+	+	+
43*	5	II(I)(III)	B	+	+++	++	++	-	+	+	++
46	3	II	B	+	+++	++	++	+	+	+	+

+++ , Nearly all; ++ , frequent; + , occasional; - , not seen.

* Pre-existing naevus.

† Cell types which were seen occasionally are shown in parentheses.

TABLE 4. Melanosomal characteristics in superficial spreading melanoma

Series no.	Level of invasion	Predominant cell type	Grade of differentiation	Melanosomes							Autophagocytosis
				Spheroidal	Ellipsoidal	Normal	Abortive	Vacuolar	Granular	Lamellar	
Superficial spreading melanoma											
1	?	III	C	+++	-	-	++	++	++	++	++
5*	5	III(IV)†	C	+++	-	-	+	+	++	+	+
7	3	II(III)(IV)	B	++	++	++	++	+	+	+	+
10	4	II/III	C	+++	+	+	++	++	++	+	+
14	4	III(IV)	C	+++	+	+	++	++	++	+	+
15	2	III(II)(IV)	C	+++	+	-	++	++	++	+	++
20*	2	II/III	C	+++	+	-	+++	++	++	+	++
25*	4	III(IV)	C	+++	+	+	++	+	++	++	++
27*	5	II(I)(IV)	B	++	++	++	++	+	+	+	+
33*	3	II/III	C	+++	-	-	++	++	++	+	+
35*	3	III(II)	C	+++	+	+	++	++	++	++	++
37	3	III(II)(IV)	B	+++	+	+	++	++	++	+	+
39	5	II	B	++	++	++	++	++	+	+	++
45	3	III(II)	C	+++	-	-	++	+	++	+	+

+++ , Nearly all; ++ , frequent; + , occasional; - , not seen.

* Pre-existing naevus.

† Cell types which were seen occasionally are shown in parentheses.

TABLE 5. Melanosomal characteristics in nodular melanoma and secondary melanoma deposits

Series no.	Level of invasion	Predominant cell type	Grade of differentiation	Melanosomes							Autophagocytosis
				Spheroidal	Ellipsoidal	Normal	Abortive	Vacuolar	Granular	Lamellar	
Nodular melanoma											
2	3	III(IV)	C	+++	-	-	+	-	++	+	+
3	5	III(II)(IV)	C	+++	-	-	++	+	++	+	+
16	4	III/IV	C	+++	+	-	+	+	++	+	++
26	4	II/III(IV)	C	++	++	+	++	+	++	+	+
38*	4	II/III	B	+++	+	+	++	+	++	++	+
41*	3	II/III	C	++	++	+	++	+	++	+	+
42	5	I/II	B	++	++	++	++	+	+	+	-
44	4	III(II)(IV)	C	+++	-	-	++	+	+	+	+
63	4	IV	C	+	+	-	+	+	+	-	-
Secondary melanoma											
31		III(II)	C	+++	+	-	++	+	++	++	+
32		III(IV)	C	+++	-	-	++	+	++	+	+
51		II(III)	B	++	++	++	++	+	++	+	+

+++ , Nearly all; ++ , frequent; + , occasional; - , not seen.

* Pre-existing naevus.

† Cell types which were seen occasionally are shown in parentheses.

melanosomes appear to be more common in superficial spreading melanoma in this series. It will be interesting to see whether this finding can be confirmed.

Fine structural appearances can therefore be classified into two broad categories. In the first the tumour cells still resemble normal melanocytes (differentiated group), whilst in the second all such resemblance is lost, and there is only poor subcellular differentiation (poorly differentiated group).

The existence of a preceding naevus and the depth of invasion of the tumour cannot be related specifically to either picture. Melanomas which develop in association with naevi can exhibit fine structural characteristics of either group and the more deeply invading tumours do not appear to contain only poorly differentiated cells.

The prognosis in patients with malignant melanomas depends on many variables, but can be conveniently related to three important features, clinical stage (spread) of the disease (Luce *et al.*, 1973), depth of invasion of primary tumour (Cochran, 1969; Clark *et al.*, 1969; McGovern, 1970; Little, 1972; Breslow, 1975), and histogenetic pattern (Clark *et al.*, 1969). However, McGovern (1976) has pointed out the overriding of histogenetic type by depth of invasion as far as prognosis is concerned.

It is too early to relate the ultrastructural appearances noted in this study with prognosis. In our original analysis we assessed the depth of invasion using the Sydney classification (McGovern *et al.*, 1973), but since then we have also reviewed the sections according to Breslow's method (Breslow, 1970), and the results are similar. There is no clear cut relationship between fine structure and depth of invasion of the primary tumour. Nevertheless, it is possible that those patients with tumours extending to Level V may have a better prognosis if the ultrastructural appearance is that of the differentiated group (e.g. patients 28, Table 3; 39, Table 4; 42, Table 5). Put another way, the 12% of patients surviving with primary tumours at Level V (Clark *et al.*, 1969) could be those with tumours having a differentiated ultrastructural appearance.

The same sort of reasoning can be applied to survival according to histogenetic patterns (Clark *et al.*, 1969). Do the few patients who survive with nodular melanoma (27% in series of Clark *et al.*) have ultrastructurally differentiated tumours? Conversely, are most of the deaths associated with the poorly differentiated pattern? Time is needed before these important questions can be answered. This series should be analysed again in 5 years, and these points investigated. If correlations are found, then there could be obvious therapeutic implications, as earlier and more aggressive treatment would seem to be indicated for patients with a poor prognosis.

Comparison of results in this study with those of previous studies

The two largest previous series are those of Cesarini, (1971) and Klug & Günther (1972). Klug & Günther (1972) distinguished two main types of melanoma cell which they termed 'Type A' and 'Type B'. Type A cells contain cigar shaped melanosomes, and Type B cells spheroidal ones. Unfortunately the occurrence of A and B cells was not formally correlated with different histogenetic patterns, but Type B cells may correspond with those noted in lentigo maligna melanoma by Mishima (1967). This was a disappointment to us as their Type A cell had appeared to correspond with our differentiated cells and their Type B cell with the poorly differentiated type.

In Cesarini's (1971) study of 100 cases of malignant melanoma there was no attempt to correlate melanosomal profiles with histogenetic types. However, his Fig. 4c does show some typical vacuolar melanosomes.

Our findings are in general agreement with those of Clark *et al.* (1972) which is encouraging as we employed their criteria and terminology. Clark *et al.* emphasized the overlap of fine structural appearances between different histogenetic types. We have verified this and support Clark's view that the ultrastructural features of lentigo maligna melanoma are sufficiently distinctive to permit its separate

identification, a view also shared by Hirone *et al.* (1971) and Lupulesco *et al.* (1973). In contrast Mishima (1967) and McGovern & Lane Brown (1969) found the melanosomes of lentigo maligna melanoma to be pleomorphic. However Mishima's observations were made at a time when superficial spreading melanoma had not been distinguished from lentigo maligna melanoma.

McGovern & Lane Brown (1969) and Anton-Lamprecht & Tilgen (1972) noted large cytoplasmic vacuoles (diameter 1–1.4 μm) in lentigo maligna melanoma cells which differ from vacuolar melanosomes. We have observed such vacuoles (Fig. 9) and have noted that they occur with similar frequency in superficial spreading and nodular melanomas and agree that they are most likely formed by lipid degeneration (Anton-Lamprecht *et al.*, 1971).

Mishima & Matsunaka (1975) recently reported that the cells of superficial spreading melanoma synthesized 'rather uniquely spheroid melanosomes, containing finely granular, densely distributed melanin particles and occasionally whirlpool-like internal structure' and recorded their diameter as 500–700 nm. We found granular and lamellar melanosomes frequently in melanomas other than superficial spreading type, and found them to be smaller (150–400 nm). Further studies of this point are necessary before firm conclusions can be drawn.

Klingmüller *et al.* (1970) failed to relate melanosomal fine structure and histogenetic type, and Jakubowicz *et al.* (1970) felt studies of melanosomal morphology in melanoma to be of limited value, in that differences in melanosomal structure were insufficient to permit their use in diagnosis or differential diagnosis.

Curran & McCann (1976) compared the fine structure of benign pigmented naevi with that of malignant melanoma. They concluded that a range of fine structural abnormalities distinguished malignant melanomas from naevi. Those affecting melanosomes were most constantly present. Although they did not classify the malignant melanomas according to the histogenetic types used here, they found no firm ultrastructural evidence to support the existence of two types of melanomas. They concluded that electron microscopy provided no additional help to light microscopy in determining the diagnosis and prognosis in malignant melanoma.

Fine structure and the pathogenesis of malignant melanoma

Mishima (1967) originally proposed that there were differences between the oncogenesis of lentigo maligna melanoma and other melanomas. If melanosomal fine structure reflects these differences, then our study would support this part of his hypothesis. However, on ultrastructural grounds, we could find no evidence to support the recent modification of his concept (Mishima & Matsumaka, 1975) that all superficial spreading and nodular melanomas develop from junctional naevi.

Mishima's view that melanomas developing from naevi contain characteristic melanosomes is supported only by Hirone *et al.* (1971) who found that granular melanosomes were confined to naevocytic melanomas. However, they examined only two such specimens. In this study, granular melanosomes were seen in all histogenetic types of malignant melanoma and could not be related to pre-existing naevi.

The histogenetic typing favoured by many workers (and used in this paper) stems from the studies of Clark (1967) and McGovern (1970). Clark *et al.* (1972) found naevi associated with all three histogenetic types and considered that melanocytes, whether involved in a naevus or as part of the normal intraepidermal melanocytic population, may equally be affected by the appropriate carcinogens.

It is difficult to be sure of the nature of any pre-existing pigmented lesion, and the historical criteria used in this study were probably over-strict. Nevertheless, that pre-existing naevi were similarly frequent in all histogenetic types supports the view of Clark *et al.* (1972) as does the absence of a distinctive and melanosomal pattern in melanomas developing in association with pre-existing naevi.

There are undoubtedly relatively simply clinical and histological features which distinguish the

histogenetic types of malignant melanoma and provide the *raison d'être* of the Sydney classification. Lentigo maligna melanoma is slow growing and relatively benign; whereas the other types of melanoma are more aggressive (Clark, 1967). Paralleling the clinical differences, lentigo maligna melanoma retains relatively normal melanosome synthesis whereas the more aggressive tumours form few normal melanosomes.

Melanosomal fine structure is under genetic control (Moyer, 1963). In mice, mutant alleles cause disorientation of melanosomal fibres associated with a reduction of fibre cross-linking. Although the carcinogenic stimulus in malignant melanoma remains unknown, a viral cause has been suggested (Birkmayer *et al.*, 1974). However speculative, it seems possible that the disorganisation of melanosomal structure, noted here in superficial spreading and nodular melanomas, could be due to a somatic mutation or a *de facto* mutation consequent upon viral incorporation into the genome.

The contrasting biological behaviour, histology and fine structure of lentigo maligna melanoma, when compared with other types of melanoma, suggest a different mode of carcinogenesis in lentigo maligna melanoma. However, it remains a matter of conjecture whether this is due to a different carcinogenic agent or to a more orderly and conditioned host response to the same agent.

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REFERENCES

- ANTON-LAMPRECHT, I., SCHNYDER, U.W. & TILGEN, W. (1971) Das 'Stade ephelide' der melanotischen Präcan-cerose. *Archiv für Dermatologische Forschung*, **240**, 61.
- ANTON-LAMPRECHT, I. & TILGEN, W. (1972) Zur Ultrastruktur der melanotischen Präcancerose. *Archiv für Dermatologische Forschung*, **244**, 264.
- BIRKMAYER, G.D., BALDA, B.R. & MILLER, F. (1974) Oncorna-viral information in human melanoma. *European Journal of Cancer*, **10**, 419.
- BLEEHEEN, S.S., HARTLEY, L. & SENIOR, J. (1976) Lentigo-maligna melanoma: an ultrastructural study. *British Journal of Dermatology*, **95**, Suppl. **14**, 14.
- BRESLOW, A. (1970) Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous malignant melanoma. *Annals of Surgery*, **172**, 1902.
- BRESLOW, A. (1975) Tumor thickness, level of invasion and node dissection in stage I cutaneous melanoma. *Annals of Surgery*, **182**, 572.
- CESARINI, J.P. (1971) Recent advances in the ultrastructure of malignant melanoma. *Revue Europeene d'études Cliniques et Biologiques*, **16**, 316.
- CLARK, W.H. (1967) A classification of malignant melanoma in man correlated with histogenesis and biologic behaviour. In: *Advances in Biology of Skin. Vol. VIII. The Pigmentary System* (Ed. by W. Montagna and F. Hu), p. 621. Pergamon Press, Oxford.
- CLARK, W.H., JR., FROM, L., BERNARDINO, F.A. & MIHM, M.C. (1969) The histogenesis and biologic behaviour of primary human malignant melanoma of skin. *Cancer*, **29**, 705.
- CLARK, W.H., JR., HEGGELER, B. & BRETTON, R. (1972) Electron microscopic observations of human cutaneous melanomas correlated with their biologic behaviour. In: *Melanoma and Skin Cancer* (Ed. by W.H. McCarthy), p. 121. Government Printer, V.C.N. Blight, Sydney.
- COCHRAN, A.J. (1969) Histology and prognosis in malignant melanoma. *Journal of Pathology*, **97**, 459.

- CURRAN, R.C. & MCCANN, B.G. (1976) The ultrastructure of benign pigmented naevi and melanocarcinomas in man. *Journal of Pathology*, **119**, 135.
- HIRONE, T., NAGAI, T., MATSUBARA, T. & FUKUSHIRO, T. (1971) Human malignant melanomas of the skin and their pre-existing conditions. In: *Biology of Normal and Abnormal Melanocytes* (Ed. by T.Kawamura, T.B. Fitzpatrick and M.Seiji), p. 329. University Park Press, Baltimore.
- HUNTER, J.A.A., PATERSON, W.D. & FAIRLEY, D.J. (1978) Human malignant melanoma. Melanosomal polymorphism and the ultrastructural dopa reaction. *British Journal of Dermatology*, in press.
- HUNTER, J.A.A., ZAYNOUN, S., PATERSON, W.D., MACKIE, R.M., COCHRAN, A.J. & BLEEHAN, S.S. (1976) Malignant melanoma. A comparison of cellular fine structure in invasive nodules of different histogenetic type. *British Journal of Dermatology*, **95**, Suppl. **14**, 13.
- JAKUBOWICZ, K., DABROWSKI, J., BICZYNSKI, W. & WALSKI, M. (1970) Ultrastruktur der Melanosomen in Melanomalignom. *Dermatologische Monatsschrift*, **156**, 299.
- KARNOVSKY, M.J. (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology*, **27**, 137A.
- KLINGMÜLLER, G., REUTER, G., RODERMUND, O.E. & SCHMOECKEL, CH. (1970) Die Submikroskopie des malignen Melanoms. *Dermatologische Monatsschrift*, **156**, 308.
- KLUG, H. & GÜNTHER, W. (1972) Ultrastructural differences in human malignant melanoma. An electron microscopic study. *British Journal of Dermatology*, **86**, 395.
- KOPE, A.W., BART, R.S. & RODRIGUEZ-SAINS, R.S. (1977) Malignant melanoma: a review. *Journal of Dermatologic Surgery and Oncology*, **3**, 41.
- LITTLE, J.H. (1972) Histology and prognosis in cutaneous malignant melanoma. In: *Melanoma and Skin Cancer* (Ed. by W.H.McCarthy), p. 107. Government Printer, V.C.N.Blight, Sydney.
- LUCE, J.K., MCBRIDE, C.M. & FREI, E. (1973) Melanoma. In: *Cancer Medicine* (Ed. by J.F.Holland and E.Frei), p. 1823. Lea and Febiger, Philadelphia.
- LUPULESCU, A., PINKUS, H., BIRMINGHAM, D.J., USNDEK, H.E. & POSCH, J.L. (1973) Lentigo maligna of the fingertip. Clinical, histologic and ultrastructural studies of two cases. *Archives of Dermatology*, **107**, 717.
- MCGOVERN, V.J. (1970) The classification of melanoma and its relationship with prognosis. *Pathology*, **2**, 85.
- MCGOVERN, V.J. (1976) Prognostic features in melanoma. In: *Malignant Melanoma. Clinical and Histological Diagnosis* (Ed. by V.J.McGovern), p. 121. John Wiley and Sons, New York.
- MCGOVERN, V.J. & LANE BROWN, M.M. (1969) *The Nature of Melanoma*, p. 100. Charles C. Thomas, Springfield, Illinois.
- MCGOVERN, V.J., MIHM, M.C., BAILLY, C., BOOTH, J.C., CLARK, W.H., COCHRAN, A.J., HARDY, E.G., HICKS, J.D., LEVENE, A., LEWIS, M.G., LITTLE, J.H. & MILTON, G.W. (1973) The classification of malignant melanoma and its histologic reporting. *Cancer*, **32**, 1446.
- MISHIMA, Y. (1960) Melanosis circumscripta praecancerosa (Dubreuilh)—a non-nevoid premelanoma distinct from junction naevus. *Journal of Investigative Dermatology*, **34**, 361.
- MISHIMA, Y. (1967) Melanocytic and nevocytic malignant melanomas: cellular and subcellular differentiation. *Cancer*, **20**, 632.
- MISHIMA, Y. & MATSUNAKA, M. (1975) Pagetoid premalignant melanosis and melanoma: differentiation from Hutchinson's melanotic freckle. *Journal of Investigative Dermatology*, **65**, 434.
- MOYER, F.H. (1963) Genetic effects on melanosomal fine structure and ontogeny in normal and malignant cells. In: *The Pigment Cell. Molecular, Biological and Clinical Aspects* (Ed. by M.Gordon). Annals of the New York Academy of Sciences, Vol. 100, p. 584.
- ZELICKSON, A.S. (1967) Melanocyte, melanin granule and Langerhans cell. In: *Ultrastructure of Normal and Abnormal Skin* (Ed. by A.S.Zelickson), p. 163. Lea and Febiger, Philadelphia.

Acid hydrolases in blister fluid

I. CHARACTERIZATION AND QUANTIFICATION OF ACID PHOSPHATASE

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SUMMARY

Acid phosphatase has been characterized and quantified in human suction blister fluid, interstitial fluid and serum. The acid phosphatases of suction blister fluid and serum showed differences in their pH activity curves, Michaelis-Menton constants, heat stabilities and sensitivities to inhibition by tartrate and fluoride. The behaviour of the interstitial fluid enzyme was intermediate between these two.

The levels of activity in blister fluid and serum were very similar, both being more than twice that of interstitial fluid.

These results suggest that the acid phosphatase activity in suction blister fluid is derived largely from the overlying epidermis rather than from serum.

The extent to which lysosomal enzymes participate in the sequence of events termed 'inflammation' has been the subject of considerable speculation. Although most workers agree that acid hydrolases do play a significant role in the inflammatory process, and some authors have suggested that their release may be the initial event (Johnson & Daniels, 1969), it has proved exceedingly difficult to obtain real evidence to support these ideas.

The major obstacle in the demonstration of lysosomal rupture *in vivo* is the fragility of the lysosomal membrane. Attempts to separate 'free' from 'particle-bound' hydrolase activity invariably lead to the release of substantial amounts of free enzyme during the fractionation procedure, especially in mechanically tough tissues such as epidermis. We are, therefore, exploring an alternative approach, namely the measurement of the hydrolase activity released into suction blister fluid before and after the experimental induction of inflammation. This method has already been employed successfully in the investigation of prostaglandins and other inflammatory mediators (Black *et al.*, 1977). However, if applied to acid hydrolases, certain additional factors must be considered. These include the following:

- (1) The background of plasma-derived hydrolase activity in the blister must be low, or at least must be biochemically distinguishable from that derived from epidermis.
- (2) The process of blister formation *per se* must not release an unduly large proportion of the hydrolase activity from the overlying epidermis.

Surprisingly, very little data regarding acid hydrolases in blister fluid has been reported in the literature. In order to justify the above assumptions, it has therefore been necessary to characterize and quantify these enzymes in blister fluid, in particular to obtain a direct comparison with the corresponding enzymes in interstitial fluid and serum. This preliminary work is presented in the first three papers in this series; later communications will describe the effects of UVR on the hydrolase levels of blister fluid in normal skin and in certain dermatoses.

The present report deals with the best-known of the ester hydrolases, acid phosphatase (EC 3.1.3.2). This is, in most tissues, primarily a lysosomal enzyme. In liver 70–80% of its activity is confined to the lysosomes, the remainder being distributed throughout the cytoplasm (de Duve & Berthet, 1954). Mishima (1964) demonstrated both lysosomal and non-lysosomal activity in human skin, and more recently two distinct forms of acid phosphatase have been isolated from epidermis and characterized (Miyagawa *et al.*, 1974, 1977). Although this enzyme has frequently been employed as a 'marker' for the histochemical identification of lysosomes, Mier *et al.* (1976) have shown that the distribution in epidermis in fact corresponds more closely to that of the keratinosomes than that of the classical lysosomes.

MATERIALS AND METHODS

Suction blister formation

Blisters were raised on the forearms of healthy volunteers using suction cups made of transparent plastic allowing continuous observation of the blister formation. The inner diameter of the cup was 4 cm and there was a bore of 12 mm in diameter in the adaptor plate. Negative pressure was provided by an electric vacuum pump. Usually 2 or 3 cups coupled in series were used. The suction pressure was 300 mmHg when putting the cups on the skin; this was slowly raised to 400–450 mmHg over a few minutes and again lowered to 300 mmHg just at the moment when small blisters appeared. The small blisters coalesced and formed a bulla containing 0.5–0.8 ml fluid. The suction blister formation was carried out at room temperature (20–22°C); the required suction time was usually 1.5–2 h.

Collection of interstitial fluid

After collecting the suction blister fluid, the epidermis was removed by lifting the roof of the bulla and cutting it off at the border. The cups were then placed in such a manner that the bore in the adaptor plate just covered the area of the removed epidermis. The suction pressure was raised to 300–350 mmHg and after 1 h, 0.5–1 ml interstitial fluid was collected in each suction cup.

Protein and cellular content of the fluid

In determining the protein concentration of serum, interstitial fluid and blister fluid a Biuret reaction was used. A cellulose acetate electrophoresis was made of the samples and the fractions were determined densitometrically. These clinical chemical analyses were performed at the Department of Clinical Chemistry, Rikshospitalet, Oslo.

The amount of red and white blood cells was checked by means of a Colter Counter Celloscope at the same department.

Chemicals

4-Methylumbelliferone (4-MU) and 4-methylumbelliferyl phosphate (4-MU phosphate) were purchased from the Sigma Chemical Company.

Assay procedure

Calibration curves were constructed with a standard solution of 4-MU (10^{-6} mol/l) in carbonate-bicarbonate buffer, pH 10.5, and the fluorescence read with a SPF-125 Spectrophotofluorometer

(American Instrument Company). The fluorescence was read with maximum exciting wavelength 362 nm and maximum emitting wavelength 450 nm. Linear calibration curves were obtained for 4-MU concentrations of 0.1–4 nmol/l (investigated range).

The reaction was initiated by the addition of 0.1 ml of serum, interstitial fluid or blister fluid to 0.1 ml of buffered 4-MU phosphate (1 mmol/ml). After incubation for 30 min at 37°C the reaction was stopped by the addition of 1 ml of carbonate–bicarbonate buffer (0.2 mol/l, pH 10.5). Corresponding blanks (buffered substrate and serum, interstitial fluid or blister fluid) were incubated with each assay. Duplicate samples and blanks were employed throughout. The fluorescence of all samples was measured within a few minutes after terminating the reaction. After subtraction of the blanks, the enzymatic activity was calculated as nmoles of 4-MU liberated per min per ml serum, interstitial fluid or blister fluid.

Acetate (0.2 mol/l), glycine-HCl (0.2 mol/l) and citrate (0.1 mol/l) buffers showed no advantage over 0.04 mol/l lactate buffer, so the last was used throughout.

Effects of reaction variables

The effect of the incubation period was investigated from zero up to 90 min at pH 4.3 for blister fluid, interstitial fluid and serum, and the enzymatic activity plotted as a function of time. The linearity of the reaction was verified by diluting samples with water in the range 1:1 to 1:10 and conducting assays as described above. pH curves were made in the pH range 3.6–6.6. The enzymatic activity was plotted as a function of pH.

A series of 4-MU phosphate concentrations in the range 0.4–2 mmol/ml was incubated for 30 min at pH 4.3 for blister fluid, interstitial fluid and serum. Lineweaver & Burk plots (reciprocal of the reaction velocity plotted as a function of the reciprocal of substrate concentration) were made and the Michaelis–Menton constant (K_m) obtained by extrapolation.

Samples of serum, interstitial fluid and blister fluid were incubated anaerobically at 56°C for 5 and 30 min. pH measurements were taken before and after incubation. The enzymatic activity was in each case compared with non-thermal inactivated samples.

The following inhibitors were added to the substrate; 5 mmol/l Na tartrate, 5 mmol/l NaF, 5 mmol/l $MgCl_2$, 5 mmol/l $CaCl_2$, 5 mmol/l KCl. The results were compared with controls without inhibitors.

RESULTS

Suction blister formation and collection of interstitial fluid

Small blisters appeared after about 45 min in elderly patients and after 60 min in younger ones. The average suction time was 1.5 h for elderly and 2 h for younger patients. No correlation of enzyme activity with time needed for bulla formation was to be found (plus or minus half an hour).

Petechiae were rather rare when suction pressure was raised not too fast and not higher than 400–450 mmHg. Blisters easily ruptured if the suction pressure was not lowered at the moment they appeared. Bleeding occurred during the collection of interstitial fluid if the suction pressure was too high. No visible scarring was produced.

Protein and cellular content of the fluid

The total protein concentration and the various protein fractions found for suction blister fluid and interstitial fluid are listed in Table 4.

The number of erythrocytes varies from 0 to 10 per mm^3 for suction blister fluid and from 0 to 20 per mm^3 for interstitial fluid. The total number of leukocytes per mm^3 was in the range 0–20 for suction blister fluid and 0–45 for interstitial fluid.

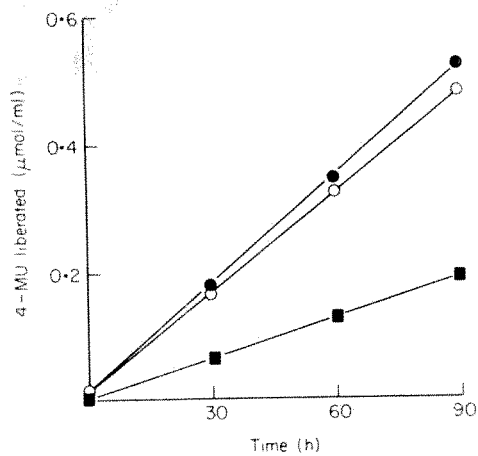


Fig 1

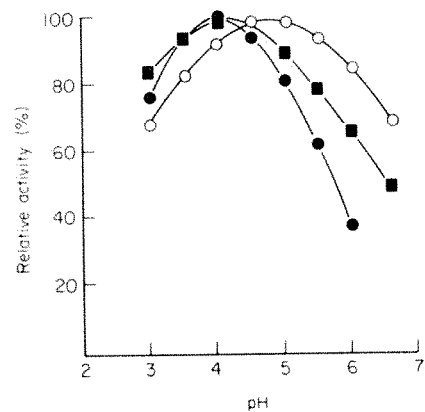


Fig 2

FIGURE 1. Progress curves showing the release of 4-MU ($\mu\text{mol/ml}$) at periods up to 90 min, in serum (○), blister fluid (●) and interstitial fluid (■). The zero values were obtained by stopping the reaction immediately, fluorometric measurements, subtraction of blank (substrate and serum, interstitial fluid or blister fluid).

FIGURE 2. The effect of pH on the activity of acid phosphatase in serum (○), blister fluid (●) and interstitial fluid (■).

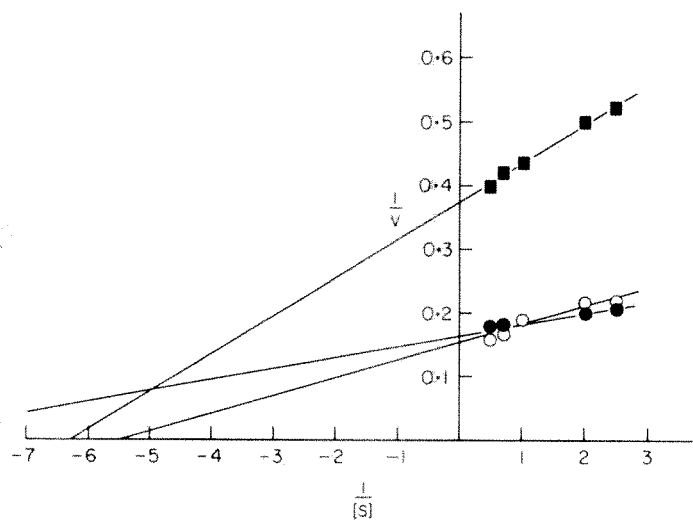


FIGURE 3. Lineweaver and Burk plots of the acid phosphatase activities of serum (○), blister fluid (●) and interstitial fluid (■). Reaction velocities are in nmol/min/ml and substrate concentrations in $\mu\text{mol/ml}$.

TABLE 1. Thermal stability of acid phosphatases at 56°C. Activity expressed as percentage of control (100%)

Heat inactivation time (min)	Residual activity		
	Blister fluid	Interstitial fluid	Serum
5	78	84	100
30	27	48	93

No significant correlation of enzyme activity with protein content or cellular content was to be found. Of course, the small amount of cells and slight variation in protein concentration must be taken into account.

Effects of incubation time and enzyme concentration

The progress curves in Fig. 1 show linear reaction rates for at least 90 min for all three samples. The reaction rate was proportional to the concentration of the sample in the dilution range 1:1 to 1:10.

Effect of pH

The pH curves in Fig. 2 show a pH optimum at 4.0 for blister fluid, 4.3 for interstitial fluid and 4.8 for serum. The pH activity curve in serum was flatter than in interstitial fluid and still flatter than the curve in blister fluid.

Effect of substrate concentration

Lineweaver & Burk plots are illustrated in Fig. 3. K_m values for serum, interstitial fluid and suction blister fluid were respectively 1.8×10^{-4} , 1.6×10^{-4} and 1.0×10^{-4} mol/l.

Heat inactivation

pH was 7.4 in blister fluid, interstitial fluid and serum just after collecting the samples. After 5 min heating at 56°C the same pH value was found in all the samples, and after 30 min heating at the same temperature, pH became 7.5 in all the samples. The enzyme activity remaining in the various samples after 5 min and 30 min at 56°C is shown in Table 1. It is seen that the blister fluid and interstitial fluid enzymes are considerably more heat-labile than the serum enzymes.

TABLE 2. Effect of various inhibitors on acid phosphatase activity. The concentration of all inhibitors in the substrate was 5 mmol/l. Activity expressed as percentage of control (100%)

Source	Residual activity				
	Na tartrate	MgCl ₂	CaCl ₂	KCl	NaF
Blister fluid	31	91	89	85	2
Interstitial fluid	42	86	86	77	3
Serum	90	95	85	86	16

TABLE 3. Quantification of acid phosphatases in suction blister fluid, interstitial fluid and serum. Activity expressed as nmol/min/ml. n = number of subjects investigated

Source	n	Mean value	Range
Suction blister fluid	(6)	5.6	(3.3-8.8)
Interstitial fluid	(5)	2.3	(1.2-3.6)
Serum	(6)	5.5	(3.1-9.2)

Sensitivity to inhibitors

Table 2 shows that $MgCl_2$, $CaCl_2$ and KCl all slightly inhibited the activity of acid phosphatases in suction blister fluid, interstitial fluid and serum. Na tartrate inhibited the activity in suction blister fluid and interstitial fluid respectively 69 and 58%, while the activity in serum was inhibited only 10%. The activity in suction blister fluid and interstitial fluid was almost totally inhibited by NaF.

Normal ranges

The activities of acid phosphatase in suction blister fluid, interstitial fluid and serum are listed in Table 3. It is seen that the mean activity of interstitial fluid is less than half that of blister fluid or serum.

DISCUSSION

Kiistala & Mustakallio (1967) showed by a biopsy specimen removed immediately after blister formation that detachment of the epidermis from the dermis at the dermo-epidermal junction resulted in the formation of a subepidermal blister. Microscopically they found that suction blistering started on the tops of the dermal papillae. When the separation had proceeded over the epidermal ridges, the eccrine ducts and hair follicles were broken. The sebaceous glands remained in the dermis. All blisters were cleanly sub-epidermal. Electronmicroscopically they showed that the level of separation was between the basal cell membrane, the hemidesmosomes and the basal lamina. In the same study they found mild oedema in the upper part of the dermis; otherwise the dermal alterations appeared mild and there was no histological sign of activation of lysosomal hydrolases.

TABLE 4. The adult normal ranges for total serum protein and the various fractions at the Department of Clinical Chemistry, Rikshospitalet, Oslo, and the values of total protein and the various fractions in suction blister fluid and interstitial fluid. The mean value of 4 assays is expressed as g/l for suction blister fluid and interstitial fluid

Protein	Serum	Suction blister fluid		Interstitial fluid	
		Mean value	Range	Mean value	Range
Total protein	63-80	18	(14-26)	21	(14-32)
Albumin	33-49	9	(6-13)	11	(7-15)
Alpha 1-glob.	2-4	1	(1)	1	(1-2)
Alpha 2-glob.	4-8	1	(1-2)	2	(1-3)
Beta-glob.	6-10	2	(2-3)	3	(2-4)
Gamma-glob.	9-17	4	(3-6)	5	(3-9)

Cellulose acetate electrophoresis of suction blister fluid and interstitial fluid showed a 'serum like' pattern qualitatively, but the total content was about 1/4 of that of serum. These findings correspond well to those reported by Kiistala (1971), who found that the blister fluid-to-serum ratios for various proteins investigated reveal that blister fluid is principally an ultrafiltrate of serum. Our findings indicate that the same conclusion could be drawn for what we in this study call interstitial fluid. The latter fluid also has to pass the basal cell membrane before collection.

Regarding the properties of the acid phosphatases from the various sources, it is quite clear that the enzyme in blister fluid is by no means identical to that in serum: the pH optima, Km values, heat stability and sensitivity to inhibitors such as tartrate all show distinct differences. It is of interest that, in each case, the properties of the interstitial fluid acid phosphatases are intermediate between those of blister fluid and serum. These data suggest that the enzyme in interstitial fluid is in part derived from serum, and in part from local cellular sources; the blister fluid enzyme, on the other hand, is presumably derived mainly from the overlying epidermis. The presence of multiple molecular species, however, (Barret, 1972) indicates the need for caution in the interpretation of such parameters; it is conceivable that differences in apparent Km values and other properties could result from the preferential diffusion of individual isoenzymes.

Turning to the quantitative aspects of our findings, the much higher level of phosphatase activity in blister fluid (5.6 nmol/min/ml) compared with interstitial fluid (2.3 nmol/min/ml) supports the view that at least the major part of the blister fluid activity is indeed derived from epidermis. Assuming the difference between these figures (i.e. 3.3 nmol/min/ml) to be leakage from the bulla roof, estimating the weight of this as about 20 mg, and employing the figure of 880 nmol/min/g from the acid phosphatase of human epidermis (Mier & Van Den Hurk, 1975), we may calculate this leakage to represent 10–15% of the total activity of the overlying epidermis. It seems likely that this surprisingly high figure, in the absence of overt epidermal damage, indicates a rapid diffusion of the cytoplasmic component rather than originating from lysosomal sources. In either case, however, it is reasonable to suppose that any substantial degree of lysosomal rupture prior to blister formation will result in an increased level of acid phosphatase in the blister fluid.

In summary, therefore, our present findings suggest that acid phosphatase may indeed be a suitable enzyme to use as an index of lysosomal labilisation. Papers to follow will describe our preliminary investigations on the glycoside hydrolases (EC 3.2) and the peptide hydrolases (EC 3.4); further communications will deal with the effects of UVR on the release of these enzymes from normal and pathological epidermis.

REFERENCES

- BARRETT, A.J. (1972) Lysosomal enzymes. In: *Lysosomes: a Laboratory Handbook* (Ed. by J.T.Dingle), p. 87. North-Holland Publishing Co., Amsterdam.
- BLACK, A.K., EADY, R., HENSBY, C.N., PLUMMER, N.A. & GREAVES, M.W. (1977) A new method of recovering inflammatory mediators from human skin. *Abstracts of the Annual Meeting of the E.S.D.R.*, 1977, Amsterdam, The Netherlands.
- DE DUVE, C. & BERTHET, J. (1954) The use of differential centrifugation in the study of tissue enzymes. *International Review of Cytology*, **3**, 225.
- JOHNSON, B.E. & DANIELS, F. (1969) Lysosomes and the reactions of skin to ultraviolet irradiation. *Journal of Investigative Dermatology*, **53**, 85.
- KIISTALA, U. (1971) Proteins in suction blisters of human skin. Paper read at *The First Annual Meeting of the E.S.D.R.*, Noordwijk aan Zee, The Netherlands.
- KIISTALA, U. & MUSTAKALLIO, K.K. (1967) Dermo-epidermal separation with suction. Electron microscopic and histochemical study of initial events of blistering on human skin. *Journal of Investigative Dermatology*, **48**, 466.
- LAZARUS, G.S. & HATCHER, V.B. (1975) Lysosomes and the skin. In: *Lysosomes in Biology and Pathology* (Ed. by J.T.Dingle and R.T.Dean), vol. 4, p. 111. North-Holland Publishing Co., Amsterdam.

- MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975) Lysosomal hydrolases of the epidermis. 2. Ester hydrolases. *British Journal of Dermatology*, **93**, 391.
- MIER, P.D. & VAN DEN HURK, J.J.M.A. (1976) Lysosomal hydrolases of the epidermis. 6. Changes in disease. *British Journal of Dermatology*, **95**, 271.
- MISHIMA, Y. (1964) Lysosomal and non-lysosomal acid phosphatase activity of the human skin. *Journal of Cell Biology*, **23**, 122 A.
- MIYAGAWA, T., KODA, H. & URABE, H. (1974) Chromatographic fractionation and partial characterization of acid phosphatase in guinea-pig epidermis. *Journal of Investigative Dermatology*, **63**, 476.
- MIYAGAWA, T., ANAI, M. & URABE, H. (1977) Purification and characterization of guinea-pig epidermal acid phosphatase. *British Journal of Dermatology*, **96**, 263.

Acid hydrolases in blister fluid

II. CHARACTERIZATION AND QUANTIFICATION OF GLYCOSIDE HYDROLASES

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SUMMARY

Seven glycoside hydrolases have been investigated in suction blister fluid, interstitial fluid and in serum. Six of these have been characterized; no differences could be demonstrated between the corresponding enzymes from the various sources. The remaining enzyme (β -glucosidase) was not found.

Quantitative data suggest that 2 enzymes (β -acetylglucosaminidase and β -glucuronidase) diffuse freely from the epidermis into blister fluid, whereas 4 (α -glucosidase, α - and β -galactosidase and α -mannosidase) are almost entirely retained in the roof of the bulla.

Glycoside hydrolases (EC 3.2) have recently received considerable attention, not only because of their importance in lysosomal processes in general but also because of their central role in storage diseases. Although most of the glycoside hydrolases are localized chiefly or exclusively in lysosomes, certain of these enzymes have been shown to occur also in other organelles or in the cytoplasm (Barrett, 1972).

This paper describes preliminary studies on the enzymes of blister fluid, interstitial fluid and serum analogous to those reported on acid phosphatase in the preceding communication (Volden, 1978).

MATERIALS AND METHODS

The suction blister technique and the collection of interstitial fluid is described in a previous report (Volden, 1978). The work of Mier and Van Den Hurk (1975) on glycosidases of epidermis indicated that the use of 4-methylumbelliferyl (4-MU) glycosides provided considerably greater sensitivity over alternative methods currently available. The fluorogenic substrates, substrate concentrations, and pH values used for the assay in this study are listed in Table 1.

Assay procedure

The assay procedure was the same as previously described with a few minor alterations. The substrates were dissolved in dimethyl sulphoxide (DMSO) at 50 mmol/l; for routine assay this stock

TABLE 1. Substrates and pH values used for the assay of the individual glycoside hydrolases

EC number	Enzyme	Substrate	Source	pH of assay
3.2.1.20	α -glucosidase	4-MU- α -D-glucoside	Sigma	5.5
3.2.1.21	β -glucosidase	4-MU- β -D-glucoside	Sigma	
3.2.1.22	α -galactosidase	4-MU- α -D-galactoside	Koch-Light	3.6
3.2.1.23	β -galactosidase	4-MU- β -D-galactoside	Sigma	3.4
3.2.1.24	α -mannosidase	4-MU- α -D-mannoside	Koch-Light	4.6
3.2.1.30	β -acetylglucosaminidase	4-MU-N-acetyl- β -D-glucosaminide	Sigma	4.0
3.2.1.31	β -glucuronidase	4-MU- β -D-glucuronide	Sigma	3.4

solution was diluted with lactate buffer (0.04 mol/l) to a final substrate concentration of 1 mmol/l. Contamination with free 4-MU, indicated by alkaline fluorescence, was removed by extracting the solution with an equal volume of ether; remaining traces of ether were removed by passing a current of air through the solution. The incubation time was 60 min at 37°C. The reaction was terminated by the addition of carbonate-bicarbonate buffer (0.2 mol/l, pH 10.2), and fluorescence ($\lambda_{\text{ex}} = 362$ nm, $\lambda_{\text{em}} = 450$ nm) was read within a few minutes.

Investigations

The linearity of the reactions was investigated with respect to time (0–90 min) and to enzyme concentration (1:1–1:10 sample dilution). Other variables studied included the effects of pH, substrate concentration, heat inactivation (56°C for 5 and 30 min), possible activators or inhibitors (DMSO, tartrate, MgCl_2 , CaCl_2 , KCl and NaF) and the use of alternative buffer systems (glycine-HCl, 0.2 mol/l and citrate, 0.1 mol/l).

The enzyme activity was studied with respect to protein concentration and cellular content of the fluid (Volden, 1978).

RESULTS

General

The 7 investigated glycosides were hydrolysed at different rates. No hydrolysis of 4 MU- β -D-glucoside occurred with any sample; the enzyme β -glucosidase is thus apparently absent from blister fluid, interstitial fluid and serum, and will not be discussed further. The remaining glycosides were all hydrolysed at sufficient rate to permit accurate quantification under the conditions described, even the least active enzyme of these (α -galactosidase) yielding a reaction product equivalent to about 4 times the total blank.

Neither of the alternative buffer systems (glycine-HCl and citrate) offered any advantage over lactate: the last was therefore employed in the following studies.

No correlation of any of the investigated enzymes with protein concentration or number of cells in suction blister fluid and interstitial fluid was found. The small change in protein concentration and small number of cells observed must, of course, be taken into account. Subsequent enzyme measurements did not show any correlation with time taken to bulla formation (plus or minus half an hour).

Incubation time and enzyme concentration

The rates of hydrolysis were in all cases linear with time up to at least 90 min. Activity was in general proportional to enzyme concentration over the dilution range 1:1–1:10; in the cases of α -glucosidase

and α -galactosidase, however, dilutions beyond 1:5 and 1:2 respectively yielded insufficient activity for accurate measurement.

Effect of pH

The pH optima of the various glycoside hydrolases are listed in Table 2. No significant differences were found between the enzymes from blister fluid, interstitial fluid or serum.

Effect of substrate concentration

Variation of enzyme activity with concentration of substrate followed the Michaelis-Menten equation for all substrates over the range of concentrations 0.05–2.0 mmol/l. The Michaelis-Menten constants derived by the method of Lineweaver and Burk are listed in Table 3. Similar values were obtained in blister fluid, interstitial fluid and serum.

TABLE 2. pH optima of glycosidases of blister fluid, interstitial fluid and serum

Enzyme	Investigated pH range	Blister fluid	Interstitial fluid	Serum
α -glucosidase	3.0–6.6	5.5	5.5	5.5
α -galactosidase	3.1–6.3	3.6	3.6	3.6
β -galactosidase	2.7–6.0	3.0–3.6	3.0–3.6	3.0–3.6
α -mannosidase	3.5–7.2	4.0–4.8	4.0–4.8	4.0–4.8
β -acetylglucosaminidase	3.0–5.1	4.0	4.0	4.0
β -glucuronidase	2.0–5.1	3.4	3.4	3.4

Effect of heat inactivation

The thermal stabilities of the various glycosidases are listed in Table 4. Most enzymes showed a rapid initial loss of activity, followed by a relatively gradual decline; β -acetylglucosaminidase lost all activity within 5 min. Good agreement was again found between the corresponding enzymes from each of the three sources.

Activators and inhibitors

The effects of DMSO (2%, 4% and 8% w/v) are shown in Table 5. β -Acetylglucosaminidase was the only enzyme to show appreciable inhibition by this compound; since DMSO is present (2%) in the

TABLE 3. Michaelis-Menten constants of blister fluid, interstitial fluid and serum for the 4-MU glycosides. All values are in mmol/l

Enzyme	Blister fluid	Interstitial fluid	Serum
α -glucosidase	0.42	0.37	0.33
α -galactosidase	0.85	0.84	0.73
β -galactosidase	0.52	0.48	0.46
α -mannosidase	0.58	0.57	0.61
β -acetylglucosaminidase	0.23	0.21	0.27
β -glucuronidase	0.19	0.17	0.21

TABLE 4. Thermal stability of glucosidases of blister fluid, interstitial fluid and serum at 56°C. Activity expressed as percentage of control (100%)

Enzyme	Blister fluid		Interstitial fluid		Serum	
	5 min	30 min	5 min	30 min	5 min	30 min
α -glucosidase	92	50	90	38	86	48
α -galactosidase	65	55	78	60	73	58
β -galactosidase	18	13	25	17	26	16
α -mannosidase	75	45	83	42	76	46
β -acetylglucosaminidase	1	1	1	0	0	0
β -glucuronidase	96	84	99	84	98	87

TABLE 5. Effect of DMSO concentration on glucosidases of suction blister fluid. Activity expressed as percentage of control (100%)

Enzyme	Control	2% DMSO	4% DMSO	8% DMSO
α -glucosidase	100	101	102	97
α -galactosidase	100	97	90	86
β -galactosidase	†	100	89	82
α -mannosidase	100	98	94	88
β -acetylglucosaminidase	100	68	53	38
β -glucuronidase	100	99	95	90

† Not sufficiently soluble; 2% DMSO solution expressed as 100%.

TABLE 6. Effect of activators and inhibitors on glycosidases of blister fluid (BF), interstitial fluid (IF) and serum (S). The concentration of all activators and inhibitors in the substrates was 5 nmol/l. Activity expressed as percentage of control (100%)

Enzyme	Na-tartrate			MgCl ₂			CaCl ₂			KCl			NaF		
	BF	IF	S	BF	IF	S	BF	IF	S	BF	IF	S	BF	IF	S
α -glucosidase	20	16	10	99	103	93	79	86	88	89	99	101	102	100	98
α -galactosidase	99	101	98	87	89	93	97	99	101	94	98	92	93	95	94
β -galactosidase	91	87	78	79	75	81	84	85	77	89	92	101	100	91	96
α -mannosidase	87	88	83	101	96	97	86	89	85	87	85	79	102	98	93
β -acetylglucosaminidase	103	98	103	94	98	103	100	95	104	100	105	102	93	94	99
β -glucuronidase	12	14	18	87	86	78	94	91	84	88	93	82	94	88	88

TABLE 7. Activities of glycosidases of blister fluid, interstitial fluid and serum. The mean activity of 9 assays is expressed as pmol/min/ml. Range in parenthesis

Enzyme	Blister fluid	Interstitial fluid	Serum
α -glucosidase	65 (52-90)	56 (46-88)	60 (53-95)
α -galactosidase	32 (12-50)	20 (8-38)	24 (14-40)
β -galactosidase	130 (55-150)	130 (65-155)	150 (75-190)
α -mannosidase	120 (60-200)	110 (62-190)	134 (95-190)
β -acetylglucosaminidase	5200 (4100-6600)	3800 (2300-5100)	5400 (3600-6800)
β -glucuronidase	2300 (1900-2800)	1500 (1400-1700)	2800 (1800-4400)

standard assay medium, an appropriate correction factor is required for calculating normal values. Of the other substances tested (Table 6), only tartrate had significant effect, specifically inhibiting the enzymes α -glucosidase and β -glucuronidase. Once again, no differences in the behaviour of the enzymes from the various sources could be established.

Normal ranges

Although wide differences were observed in the activities of the individual enzymes (Table 7), in each case the values for blister fluid and serum were similar, rather lower values being found for interstitial fluid. No β -glucosidase activity was observed in any sample.

DISCUSSION

The only technical difficulty inherent in the assay procedure used here is the poor solubility of certain of the 4 MU-glycosides, in particular the α -galactoside and α -mannoside. Even using 2% DMSO as solubilizing agent, it is not possible to reach a substrate concentration of twice the K_m value for these two substrates; thus zero order kinetics may not be followed. Although slow non-enzymatic decomposition of certain substrates occurred, this was not sufficient to limit the accuracy of the determinations at the enzyme concentrations encountered.

Regarding the properties of the individual enzymes, our findings are in sharp contrast to those reported for acid phosphatase (Volden, 1978); the glycoside hydrolases from blister fluid, interstitial fluid and plasma are indistinguishable in their behaviour. This observation does not, of course, imply that the glycosidases in blister fluid are necessarily derived from plasma; it merely confirms the general impression from the literature that the biochemical parameters of this group of enzymes are remarkably similar regardless of the tissue of origin. Our present figures for apparent k_m values and pH optima, for example, are for the most part in good agreement with those reported by Ohkawara *et al.* (1972) for human epidermis, Mier & Van Den Hurk (1975) for guinea-pig epidermis and Kolodny & Mumford (1976) for human leukocytes.

In quantitative terms, we may again make the assumption that the epidermal contribution to the total enzymatic activity of blister fluid is indicated by the difference in activity between the blister fluid and interstitial fluid (Volden, 1978). Taking the weight of the blister roof as about 20 mg, the average volume of blister fluid as 0.65 ml, and using the values for human epidermis reported by Mier & Van Den Hurk (1976), the percentage of each enzyme which leaks into the blister fluid may be calculated. Such calculations lead to an interesting conclusion; in the cases of 4 enzymes (α -glucosidase, α -galactosidase, β -galactosidase and α -mannosidase) this leakage is less than 5% of the activity

originally present in the intact epidermis, whereas in the cases of the other two (β -acetylglucosaminidase and β -glucuronidase) it seems that about half of the epidermal activity has diffused into the blister fluid. This conclusion is wholly compatible with reports that substantial proportions of the latter two enzymes may be located outside the lysosome (reviewed by Barrett, 1972).

Returning to the use of suction blisters as a model for the study of lysosomal hydrolases in inflammation (Volden, 1978) our present findings make it clear that of this group of enzymes α -glucosidase, α -galactosidase, β -galactosidase and α -mannosidase are the logical candidates for providing an index of lysosomal labilization. The following report will describe similar investigations regarding the peptide hydrolases (EC 3.4).

REFERENCES

- BARRETT, A.J. (1972) Lysosomal enzymes. In: *Lysosomes: a Laboratory Handbook* (Ed. by J.T.Dingle), p. 46. North Holland Publishing Co., Amsterdam.
- KOLODNY, E.H. & MUMFORD, R.A. (1976) Human leucocyte acid hydrolases: Characterization of eleven lysosomal enzymes and study of reaction conditions for their automated analysis. *Clinica Chimica Acta*, **70**, 247.
- MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975) Lysosomal hydrolases of the epidermis. 1. Glycosidases. *British Journal of Dermatology*, **93**, 1.
- MIER, P.D. & VAN DEN HURK, J.J.M.A. (1976) Lysosomal hydrolases of the epidermis. 6. Changes in disease. *British Journal of Dermatology*, **95**, 271.
- OHKAWARA, A., HALPRIN, K.M., TAYLOR, J.R. & LEVINE, V. (1972) Acid hydrolases in the human epidermis. *British Journal of Dermatology*, **83**, 450.
- VOLDEN, G. (1978) Acid hydrolases in blister fluid. 1. Characterization and quantification of acid phosphatase. *British Journal of Dermatology*, **98**, 273.

HLA C and D antigens associated with psoriasis

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SUMMARY

The frequency of two newly defined HLA antigens, HLA CT7 and DMA, was found to be greatly increased in patients with psoriasis. These two antigens and those previously found to be associated with the disease, B13, BW16 and BW17, frequently occurred together. The disease may be primarily associated with HLA DMA, or with the HLA haplotypes CT7—B13/W16/W17—DMA.

More than forty human diseases have been shown to be associated with the human major histocompatibility (HLA) complex (McMichael & McDevitt, 1977). The usual finding is that one or more antigens of this highly polymorphic system are increased in frequency in the patient population. This implies that either the HLA antigen itself or a linked gene is directly involved in the disease process. If the latter hypothesis is correct the association is only seen when the disease susceptibility (D.S.) gene is in linkage disequilibrium with the marker gene, i.e. two products of two different genetic loci on the same chromosome occur together at a higher than expected frequency. As marker genes are identified that map closer to the disease susceptibility gene, so the association should become stronger.

Psoriasis is a skin disease of unknown aetiology but with a marked familial tendency (Farber *et al.*, 1974). It has been shown that the HLA antigens B13, BW16 and BW17 are increased in frequency in the patient population (Ryder & Svejgaard, 1976; Krulig *et al.*, 1975; Svejgaard *et al.*, 1974). No strong association with any HLA A locus antigen was found which suggests that the D.S. gene maps closer to the HLA B locus. It is now possible to identify products at two other HLA loci, C and D (Kiss-meyer-Nielsen, 1975).

In this study it is shown that psoriasis has associations with C and D locus products that are stronger than those previously described for B locus antigens.

METHODS

Patients

Patients attending the Stanford University Psoriasis Clinic were chosen for study. They all had classical psoriasis without arthritis. Many of the patients had a strong family history of the disease

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and almost all of them suffered from extensive involvement of their integument. Normal controls were either laboratory personnel or blood donors.

Preparation of lymphocytes

Twenty ml of venous blood was drawn into a sterile bottle containing glass beads and defibrinated. The sample was layered onto 9% Ficoll hypaque solution and centrifuged at 1300 rev/min for 35 min. The mononuclear cells at the interface were harvested, washed and resuspended in RPMI 1640 medium (Gibco-Biotech) containing 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, and 10% pooled human group A serum.

Mixed lymphocyte cultures

50,000 separated but otherwise untreated (responder) lymphocytes were mixed with 50,000 irradiated (6000 rad) stimulator lymphocytes. Triplicate cultures were prepared in round bottomed Linbro microtiter plates in 0.2 ml volumes and the mixture was incubated for 7 days. On the sixth day, 1 µCi of ³H thymidine (New England Nuclear) was added to each well and the cells were harvested the following morning with a MASH II semi-automated harvester (Microbiological Associates). The incorporation of ³H thymidine in the DNA of the lymphocytes was then counted using a Beckman scintillation counter.

HLA D typing of groups of eight to ten subjects was carried out by testing their response to irradiated HLAD homozygous lymphocyte cultures. Each response was recorded as counts per minute of ³H thymidine incorporation and was then corrected so that data collected on different occasions could be compared. The double normalization procedure described by N. Mandell and H. Festenstein (in press) was used. The counts were first expressed as a percentage of the 75th percentile response of that responder to all stimulating cells. The values obtained were then expressed as a percentage of the 75th percentile of all responses to each stimulating cell.

RESULTS

The HLA D homozygous typing cell (HTC) MA (BW17 homozygous) was found during a screening study of families with members homozygous at the HLA B locus. It was hoped to find individuals homozygous for HLA-D determinants because of the linkage disequilibrium that frequently exists between alleles at these two loci (Kissmeyer-Nielsen, 1975). HLA D homozygotes were identified by performing mixed lymphocyte reactions (MLR) between lymphocytes of family members. Irradiated MA cells failed to stimulate a response in either parent, which indicates that there is HLA D identity between the stimulating cell and one or other HLA D type of each parent. MA cells also failed to stimulate siblings' lymphocytes for the same reason.

MA was then tested as a stimulating cell in 142 individuals in the second Workshop of the Americas (Stastny, 1977). The frequency of non response to MA, which indicated sharing of her cells HLA D antigen, was 6.3%. Five of nine individuals so typed by MA showed the HLA B17 antigen. This suggests that the DMA specificity is in linkage disequilibrium with B17 though this cannot be proven without family data or larger population samples.

Because B17 was known to be increased in frequency in psoriasis thirty-nine patients were tested as responders in the MLR with MA as the stimulating cell. As controls for calculation of the double normalized relative response, each responder cell was also irradiated and used as a stimulating cell. Fig. 1 shows the corrected response of all patients to MA, compared to 25 controls. The patient group shows apparent bimodality in responsiveness to MA, one group responding normally, 58-140%; the other group, 2-42%, making a low response. The latter group is considered to share the specificity

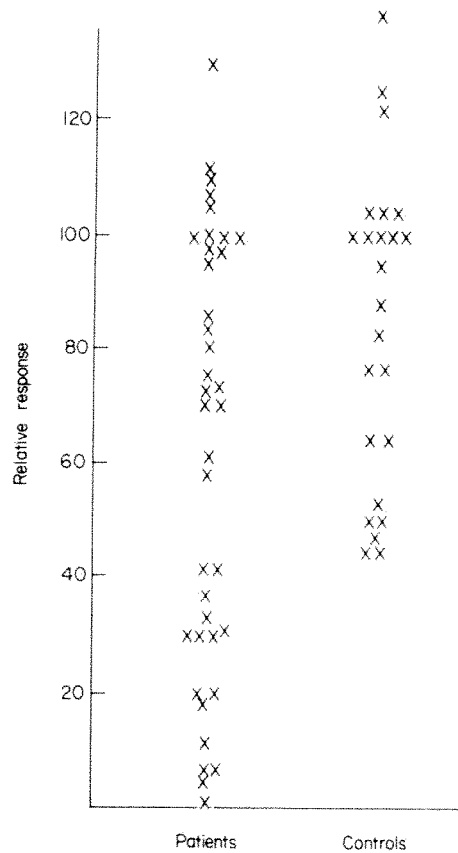


FIGURE 1. The response of 38 psoriasis patient lymphocytes and 24 healthy control lymphocytes to stimulation by irradiated MA cells in the mixed lymphocyte reaction. Relative response was calculated by the formula:

$$RR = \frac{\text{Counts per minute (c.p.m.) of } \chi}{\text{75th percentile c.p.m. of } \chi \text{ against all stimulating cells}} \times \frac{100}{\text{75th percentile response of all responding cells to stimulator MA}}$$

TABLE 1. Correlation between typing of psoriasis patients by stimulating cells MA and DT

MA+, DT+*	MA+, DT-	MA-DT+	MA-DT-	χ^2	r
9	3	3	19	10.2	0.55

* + indicates a typing (low) response and - indicates a non-typing (high) response.

r calculated from χ^2 by the formula $r = \sqrt{\frac{\chi^2}{N}}$.

TABLE 2. HLA types of psoriasis patients studied

Patient	A locus	B locus	C locus		D locus DMA
			CW4	CT7	
1	W24, W31	7, W17	—	+	+
2	2, W26	13, W16	—	+	+
3	2, 29	7, W16	—	+	+
4	11, 28	5, 8	—	+	+
5 (DT)	2, 9	W17, W16	—	+	+
6	2, 29	12, W35	+	+	+
7	1, 2	7, W17	—	+	+
8	1, 11	W16, W17	—	+	+
9	W24, W30	29, TT	—	+	+
10	11, —	W35, —	+	+	+
11	1, —	W17, —	—	+	+
12	2, —	W16, W17	—	+	+
13	2, —	W15, W40	—	—	+
14	1, W33	18, W35	—	—	+
15	n.t.	n.t.	n.t.		+
16	1, —	W17, —	—	—	+
17	1, 32	12, W17	—	+	—
18	W23, W28	12, W15	+	+	—
19	11, —	27, W35	+	+	—
20	1, 9	8, W17	+	+	—
21	W24, —	W16, W35	+	+	—
22	1, 2	12, W17	+	+	—
23	1, 2	8, W15	+	+	—
24	1, 11	W17, W35	+	+	—
25	1, 3	12, W17	—	+	—
26	2, —	W21, W39	—	+	—
27	2, 11	8, 12	—	+	—
28	2, 11	8, 12	—	+	—
29	1, 3	7, 37	—	+	—
30	3, 9	5, 13	—	+	—
31	1, —	14, W17	—	+	—
32	1, W29	12, 13	—	+	—
33	2, W32	7, W22	—	—	—
34	3, W29	7, 27	—	—	—
35	n.t.	n.t.	n.t.		—
36	n.t.	n.t.	n.t.		—
37	n.t.	n.t.	n.t.		—
38	n.t.	n.t.	n.t.		—

In this population the frequency of B13 was 3/33: 9% (7%)*, of BW16 was 6/33: 18% (5%)*; of BW17 was 13/33: 39% (6%)* of CT7 was 19-28/33: 57-84% (35%)*; of DMA 16/38: 42% (5%)*.

* Frequency in control population (Krulig *et al.*, 1975).

n.t., not tested.

defined by MA. On this basis 16 out of 38 patients (42%) have DMA. This contrasts with the healthy controls where a single distribution group was found and with the North American Workshop experience where the antigen frequency of MA was 6.3%. DMA is thus increased in psoriasis with a relative risk of 10.4 and a χ^2 of 27.6.

With this increased frequency of DMA, it is likely that homozygotes for the same specificity will

be found more frequently in the patient than in the control population. Patients' lymphocytes were therefore tested against each other in the MLR. One patient, DT, was found whose irradiated lymphocytes failed to stimulate those of several patients. The results, after double normalization, gave a pattern very similar to that found with M.A. Table 1 shows the correlation between the results found with MA and DT. In addition MA and DT were mutually non-stimulating in the MLR.

The HLA A and B types of the patients were also determined and showed, as expected, increased frequencies of HLA, BW61 and BW17 (Table 2). It has been reported by Svejgaard that there is a C locus antigen, T7, increased in frequency in psoriasis patients (Ryder & Svejgaard, 1976b). Serum 5600A, a gift from Dr Svejgaard, was therefore tested against lymphocytes from the psoriatic patients. As this serum also has specificity for HLA, CW4, five anti HLA CW4 sera (4277A, 5445A, 7168A, 7169A and 7170A) were also tested. The results are shown in Table 2. In the twenty-four patients who were not positive for CW4, 19 (79%) were positive for HLA CT7. Of these 9 out of 10 HLA BW17 positive patients, 5 out of 5 HLA BW16 positive patients, 3 out of 3 HLA B13 positive patients and 11 out of 13 HLA-DMA positive patients were HLA CT7 positive. This minimum frequency estimate compares with a normal frequency of CT7 of 35% in Caucasians in a recent study (Payne, 1977). The relative risk for developing psoriasis in HLA CT7 positive individuals is at least 7.0.

DISCUSSION

HLA products at three loci are increased in frequency in psoriasis: B13, W16 and W17, CT7 and DMA. Based on relative risk values, the D locus association seems to be the strongest. HLA DMA is a rare antigen in the normal population, but was found in 42% of psoriasis patients using the homozygous typing cell MA. The data shown in Table 2 suggest that a very strong association exists between the disease and the HLA haplotypes CT7—B13/W16/W17-D MA. Because we do not have sufficient family data we cannot calculate exact values for the linkage disequilibria that exist in these haplotypes and it is not clear whether these haplotypes are in linkage disequilibrium in the healthy population. If these haplotypes are unique to the disease population it could mean that the psoriasis gene was a recent mutation on this haplotype and has not yet been randomly distributed or that there are two or more genes responsible for the disease, which map at different sites in the HLA region. These points will be greatly clarified by the extensive study of a normal population which has been undertaken by the VIIth International HLA Workshop.

The HLA linked gene(s) involved in psoriasis could explain the familial tendency of the disease. The exact nature of the gene is unknown but the several possibilities have been extensively reviewed elsewhere (Ryder & Svejgaard, 1976; Moller, 1975; Sasazuki *et al.*, 1977; McMichael & McDevitt, 1977). The lack of demonstrable circulating autoantibodies in psoriasis patients makes it unlikely that there is simply an immune response gene that determines an autoimmune response to skin components. Also possible are the virus-HLA antigen interactions of the type described by Zinkernagel & Doherty (1974) in mice and recently by McMichael *et al.* (1977) in humans. An attractive possibility relates to the control of 3'5'-cyclic AMP (cAMP) levels by the mouse H-2 (HLA-equivalent) system (Meruelo & Edidin, 1975). Abnormal levels of cAMP have been found in psoriatic epidermis (Voorhees *et al.*, 1972) which may be related to the decreased ability of epidermal cells to synthesize cAMP after stimulation by prostaglandins (Aso *et al.*, 1975). It is also possible that the HLA system may be part of a broader system concerned in cell-cell recognition and that the recognitive activities of immunocytes have been discovered first because they are amenable to experimentation. There might, therefore, simply be a genetically determined deficiency of recognition between epidermal cells which is in linkage disequilibrium with the HLA CT7—DMA haplotype, and which allows abnormal proliferation to occur.

Because precise functions have not yet been mapped in the human major histocompatibility complex it is not possible to distinguish between these possible mechanisms. The stronger D locus association suggests that a function controlled by a locus that maps closer to HLA D than to B or C could be responsible. However, there might be more than one gene in the HLA complex involved which could result in the association with a particular HLA haplotype, CT7—DMA.

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REFERENCES

- ASO, K., ORENBURG, E. & FARBER, E.M. (1975) Reduced epidermal cyclic AMP accumulation following prostaglandin stimulation: its possible role in the pathophysiology of psoriasis. *Journal of Investigative Dermatology*, **65**, 375.
- FARBER, E.M., NALL, M.L. & WATSON, W. (1974) Natural history of psoriasis in 61 twin pairs. *Archives of Dermatology*, **109**, 207.
- KISSMEYER-NIELSEN (Ed.) (1975) *Histocompatibility Testing*. Munksgaard, Copenhagen.
- KRULIG, L., FARBER, E.M., GRUMET, F.C. & PAYNE, R. (1975) Histocompatibility antigens in psoriasis. *Archives of Dermatology*, **111**, 857.
- McMICHAEL, A.J. & McDEVITT, H.O. (1977) The association between the HLA system and disease. *Progress in Medical Genetics*, **II**, 39.
- McMICHAEL, A.J., TING, A., ZWEERINK, H.J. & ASKONAS, B.A. (1977) HLA restriction of cell mediated lysis of influenza virus infected cells. *Nature*, in press.
- MENDELL, N.R., GUPPY, D., BODMER, W. & FESTENSTEIN, H. (1977) Data management and assignment of scores to MLC data. In: *Histocompatibility Testing* (Ed. by W.F. Bodmer). Munksgaard, Copenhagen. In press.
- MERUELO, D. & EDIDIN, M. (1975) Association of mouse liver adenosine 3'5' cyclic monophosphate (cyclic AMP) levels with histocompatibility-2-genotype. *Proceedings of the National Academy of Science*, **72**, 2644.
- MOLLER, G. (Ed.) (1975) *Transplantation Reviews*, **22**, 1975.
- PAYNE, R. (1977) Unpublished data.
- RYDER, L.P. & SVEJGAARD, A. (1976a) Report from the *HLA and Disease Registry of Copenhagen*.
- RYDER, L.P. & SVEJGAARD, A. (1976b) Histocompatibility associated diseases. In: *T and B Lymphocytes in Immune Responses* (Ed. by F. Loors and G. Roelants), p. 437. J. Wiley and Sons, Chichester.
- SAZAZUKI, T., McDEVITT, H.O. & GRUMET, F.C. (1977) The association between genes in the major histocompatibility complex and disease susceptibility. *Annual Review of Medicine*, **28**, 425.
- SVEJGAARD, A., STAUB-NIELSEN, L., SVEJGAARD, E., KISSMEYER-NIELSEN, F., HJORTSHOJ, A. & ZACHARIAE, H. (1974) HLA in psoriasis vulgaris and in pustular psoriasis—population and family studies. *British Journal of Dermatology*, **91**, 145.
- VOORHEES, J.J., DUELL, E.A., BASS, L.J., POWELL, J.A. & HARRELL, E.R. (1972) Decreased cAMP in the epidermis of lesions of psoriasis. *Archives of Dermatology*, **105**, 695.

Mitoses in normal and psoriatic epidermis

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SUMMARY

The morning count of mitoses in uninvolved epidermis of psoriatics is not significantly different from normal skin. The afternoon count is higher than the morning in both, and significantly higher in uninvolved than in normal epidermis.

These results may be interpreted as favouring the existence of a non-cycling G₂ population in human epidermis. This G₂ population could be released into mitosis by the factors involved in diurnal variation, and might be larger and more labile in psoriatics than in normals. The pathogenesis and aetiology of psoriasis may be related to such a G₂ population.

Clues to the psoriatic process should exist in the clinically normal uninvolved skin of psoriatics. Since increase in mitoses is an outstanding feature of psoriatic epidermis, we believed that more mitoses should be found in uninvolved skin also, despite prior reports to the contrary. Therefore we decided to reinvestigate the problem, using adequate sampling, adequate number of matched controls, and rigorous statistical analysis.

MATERIALS AND METHODS

Volunteers older than forty were chosen, and three biopsies taken from the sacral area of each at 9 a.m. and again at 3 p.m. For at least a week before biopsy, no systemic or topical therapy was employed.

A stencil with a 5 × 8 mm rectangular opening was used to outline biopsy specimens, which were then removed under xylocaine anaesthesia, free hand with a scalpel, and split-skin thickness. Each specimen was placed in Bouin's fixative for no longer than 24 h to avoid impaired staining, then sectioned 8 µm thick and stained with H & E. Multiple sections were made from each biopsy from the 8 mm side, and alternating sections studied (under oil immersion, × 1000) omitting intervening ones. This avoided reading the same mitotic figure twice. The number of mitoses in 20 cm of surface length—corresponding to 40 sections and 5 mm length for each section—was recorded for each biopsy. All this has been described before (Rowe & Dixon, 1972).

We obtained material from thirty-three married couples, with one of the mates psoriatic and heavily involved, and the other without psoriasis. Sleep and wake times close to 11 p.m. and 7 a.m.

TABLE 1. Mitotic figures per cm of section in normal epidermis

P	A	B	C	D	E	F	P	A	B	C	D	E	F
Normal female—premenopausal older than 40							Normal female—postmenopausal						
1	0.80	0.40	0.90	4.20	1.55	5.05	10	3.70	4.75	2.80	3.60	7.80	4.00
2	1.35	1.60	1.15	20.65	12.85	11.70	11	0.35	0.40	0.50	4.20	3.30	1.95
3	2.35	1.90	4.10	11.00	6.65	4.05	12	0.75	0.55	0.75	3.65	5.20	2.60
4	2.50	3.60	3.10	0.90	2.80	5.00	13	1.05	1.05	0.45	7.85	7.90	6.55
5	3.65	3.05	2.50	3.75	3.50	3.45	14	1.00	1.00	1.45	2.00	1.00	0.70
6	3.20	3.50	2.65	8.00	5.50	3.90	15	1.15	1.15	1.30	3.55	5.40	6.90
7	1.80	2.10	5.75	3.60	9.55	7.70	16	2.40	1.35	0.45	7.35	4.00	8.00
8	3.15	3.50	3.25	3.50	2.65	5.75	17	1.55	2.65	1.95	2.30	2.00	1.40
9	4.25	3.20	2.90	14.60	6.30	4.85	18	5.05	2.70	3.85	1.00	0.55	1.25
Normal male—age 40-55							Normal male—older than 55						
19	1.80	1.75	0.95	3.65	1.30	2.15	27	1.70	2.05	2.10	0.25	0.35	0.50
20	3.25	1.15	1.35	3.10	2.60	1.60	28	1.50	2.95	1.75	0.65	0.75	1.20
21	4.65	1.35	5.05	5.65	1.70	3.30	29	3.00	2.55	1.20	1.80	2.65	2.90
22	25.40	6.30	1.70	1.65	21.50	7.65	30	1.90	3.30	4.00	3.75	4.20	4.70
23	0.65	0.25	0.20	5.20	0.70	1.45	31	0.40	0.50	0.40	0.85	0.70	0.40
24	0.50	1.35	0.45	4.95	3.85	0.65	32	0.85	0.95	0.25	3.45	0.35	1.60
25	1.90	1.85	1.45	5.65	9.60	4.00	33	3.55	2.00	2.85	8.40	11.60	8.30
26	2.45	4.35	4.75	7.45	9.65	6.95							

Patients are listed and identified by number in Column P. Columns A, B & C give results for the three biopsies taken from each patient at 9 a.m., and D, E & F for the three biopsies taken at 3 p.m. Results are expressed in mitotic figures per cm of section.

TABLE 2. Mitotic figures per cm of section in uninvolved epidermis of psoriatics

P	A	B	C	D	E	F	P	A	B	C	D	E	F
Uninvolved female—premenopausal older than 40							Uninvolved female—postmenopausal						
23'	0.50	0.70	0.85	1.65	0.10	0.25	28'	2.40	10.65	3.85	0.20	0.35	0.45
21'	0.40	3.70	0.85	3.50	0.95	0.30	29'	1.25	0.65	1.95	10.60	8.75	9.70
32'	0.65	0.85	0.45	4.95	1.80	2.25	30'	2.85	0.85	1.20	13.80	17.70	27.15
27'	1.85	0.95	1.35	7.10	4.75	8.35	33'	3.10	2.50	1.25	6.70	4.70	3.55
25'	1.65	1.95	2.00	6.95	6.80	9.35	31'	1.25	1.65	4.45	6.20	4.65	3.85
19'	4.40	2.70	2.05	12.85	9.25	9.70	20'	13.30	3.65	2.00	11.10	8.75	16.40
26'	4.10	3.80	4.55	11.90	10.55	8.30							
24'	11.80	6.80	1.40	35.55	31.60	32.70							
22'	24.05	7.15	8.70	32.10	21.40	24.75							
Uninvolved male—age 40-55							Uninvolved male—older than 55						
1'	0.80	0.20	0.65	5.20	2.85	2.80	12'	0.25	0.25	0.20	0.30	0.05	0.50
9'	1.60	0.90	1.30	20.25	12.35	10.65	16'	0.65	0.20	0.35	6.60	3.55	2.75
10'	2.15	1.25	2.55	1.30	0.50	0.65	15'	1.00	1.55	1.20	4.25	8.45	5.10
2'	2.35	1.75	2.90	0.70	5.80	8.00	13'	2.40	0.85	0.75	4.40	6.40	10.20
3'	1.35	1.60	2.55	27.85	11.10	13.05	17'	1.80	1.55	1.15	0.35	0.30	0.50
4'	2.65	3.25	1.80	15.10	23.45	22.40	5'	1.15	1.30	3.00	8.55	11.70	11.00
7'	1.65	2.70	4.10	26.30	40.10	28.20	11'	3.20	2.65	1.90	7.30	12.50	12.45
8'	4.00	6.35	4.90	14.90	5.25	0.95	18'	1.65	3.60	3.95	27.50	9.75	9.70
6'	1.65	2.35	1.35	17.00	19.70	13.95	14'	1.05	11.95	5.60	3.35	3.60	3.30

Patients are listed and identified by numbers in Column P. The primed numbers denote the psoriatic mates of normals in Table 1. Thus patient 23' in Table 2 is psoriatic mate of normal patient 23 in Table 1. Columns A, B & C give results for the three biopsies taken from each patient at 9 a.m. and D, E & F for the three biopsies taken at 3 p.m. Results are expressed in mitotic figures per cm of section.

prevailed for all. In addition each separate couple had the same actual sleep wake times, the same diet, and the same life style, so as to make the normal mate an acceptable control for the psoriatic.

Conversion factors, as shown in Table 3, were obtained by direct count and measurement. The number of basal cells per unit baseline length was the same in normal epidermis as in uninvolved epidermis of psoriatics (unpublished).

Tests of significance and computation of confidence limits require some knowledge of the distributional characteristics of the cell counts being observed. The counts were recorded for different surface lengths of epidermis. It was found that there was sufficient local variation in density of mitotic cells that one could not use a single parameter for the negative binomial to achieve uniformity of variance (our previous research had shown that the distributions were not Poisson (Rowe & Dixon, 1972)). A study of data for 0.5 cm, 1 cm, 5 cm and 10 cm where multiple counts from the same biopsy were available showed that subject variability had stabilized for sizes of 5 cm or more and that reasonable stability of variance was attainable with the transform $y = \log(x + 1.2w)$ where x is the count per centimetre and w is the number of centimetres counted. The coefficient of w was determined by minimizing the correlation between mean and variance.

RESULTS

Tables 1 and 2 show the number of mitoses per cm based on a study of 20 cm of section per biopsy. Table 1 is for normal epidermis and Table 2 for uninvolved epidermis of psoriatics. The P column identifies the patient. A, B and C represent 9 a.m. biopsies and D, E and F, 3 p.m. biopsies. In these tables, patients are separated as to age and sex. The patients are identified by a number in column P—numbers 1–33 in Table 1 and numbers 1'–33' in Table 2. Primed numbers were used in Table 2 to denote psoriatic patients. The corresponding non-primed numbered patients in Table 1 were the normal mates of the psoriatics. Thus patient 1 (Column P in Table 1) was the normal mate of psoriatic patient 1' (Column P in Table 2).

TABLE 3. Summary in counts per cm section. Sections 8 μ m thick

Patient	Sex	Age	No.	9 a.m.		3 p.m.	
				Mean count	95% Confidence interval for mean count	Mean count	95% Confidence interval for mean count
Normal	Female	40–55	18	2.4	1.7–3.2	4.9	3.5–6.0
Normal	Male	> 55	15	1.5	1.0–2.0	2.5	1.5–4.0
Uninvolved skin of psoriatics	Female	> 55	15	1.9	1.3–2.8	5.1	2.8–8.8
	Male	40–55	18	2.2	1.5–3.2	7.7	4.4–13.0

Menopause for females taken as at the age of 55.

Conversion factors obtained by direct count and measurement. The number of basal cells per unit baseline length was the same in normal epidermis as in uninvolved epidermis of psoriatics (unpublished).

$$\frac{\text{mitosis per cm section}}{8.7} = \text{mitoses per 1000 viable epidermal cells.}$$

$$\frac{\text{mitosis per cm section}}{1.76} = \text{mitoses per 100 basal cells.}$$

TABLE 4. Analysis of variance (log units)

Source	Degrees of freedom	Mean square	F	P
Between diagnostic categories	1	1.354	5.148	0.03*
Between age groups	1	1.567	5.958	0.02*
Diagnosis by age interaction	1	0.882	0.335	0.57
Error	62	0.263		
Between times of day	1	7.198	49.641	<0.0005†
Diagnosis by time interaction	1	0.769	5.303	0.03*
Age by time interaction	1	0.179	1.235	0.27
Diagnosis by age by time interaction	1	0.015	0.100	0.75
Error	62	0.145		

* Significant at the 5% level.

† Significant at the 1% level.

Table 3 shows a summary of results in count of mitoses per cm of section.

Table 4 details a repeated measure of analysis of variance (Dixon, 1975) to show magnitude of effects due to diagnostic category (normal versus uninvolved skin of psoriatics), age group and time of day.

We found no significant difference for mitotic count in (a) the normal male morning values compared to afternoon values; (b) morning values for the thirty-three normal patients as a group compared to morning values of uninvolved skin of psoriatics as a group.

Due to mitotic clustering and resultant large scatter of results, this means only that if a difference does exist in (a) and (b) above, it would be less than two fold.

However, afternoon values were significantly greater than morning values for both normal and uninvolved taken as groups of thirty-three. Also for afternoon values, the uninvolved epidermis was significantly greater than normal.

DISCUSSION

Thus there is morning to afternoon increase in mitotic count in human epidermis. This could be due either to increased entry of cells into mitosis, or to increased duration of the mitotic phase in response to the diurnal stimulus or stimuli. From work with mice (Evenson, 1963), it has been suggested that diurnal changes in mitotic count are due to increase in mitotic duration. Also work has been presented on mitotic duration in human epidermis using calculations like those of Evenson to compute results (Fisher, 1968; Fisher & Wells, 1968). The calculations used in these papers are based on reports that colchicine arrests cells in mitosis at metaphase, but does not affect the cell cycle otherwise, and on the formula:

$$\text{mitotic duration} = \frac{\text{mitotic count at time } t \text{ without colchicine}}{\text{mitotic count at time } t \text{ with colchicine}} \times t.$$

Here time (t) is 6 h or 5 h, the time during which colchicine is permitted to act on the experimental epidermis (Hooper, 1961).

As can be seen from the above formula, the fewer mitotic cells found after colchicine, the longer the mitotic duration will be calculated to be.

At the time this formula was proposed, the existence of a non-cycling G_2 population was not known. If colchicine not only arrests cells in mitosis at metaphase but also blocks the G_2 population from entering mitosis, then the mitoses after colchicine will be fewer, and hence the calculated mitotic duration greater. Actually the above formula gives mitotic duration of 90 min for normal and 4 h for psoriasis (Fisher, 1968; Fisher & Wells, 1968). However, measurements of mitotic duration using radioisotope labelling give 60 min for normal and uninvolved skin of psoriatics and about 30 min for psoriasis (Weinstein & Frost, 1968; Goodwin, Hamilton & Fry, 1974). This lends credence to the explanation proposed above.

Then, as suggested before, diurnal variation in mitotic count could be due to increased entry of cells into the mitosis portion of the cell cycle.

During part of the cell cycle, the cell nucleus takes up radioactive isotopes and becomes labelled. This has permitted separating the cell cycle into phases of measured or calculated duration. There is mitosis M, then phase G_1 with no labelling, followed by phase S with labelling and then by G_2 without labelling, leading to mitosis again.

It has been proposed that germinative cells in human epidermis are not homogeneous but consist of three distinct populations of epidermal cells. The first is called the cycling cell population. It moves actively through the cell cycle $M \rightarrow G_1 \rightarrow S \rightarrow G_2 \rightarrow M$ without interruption except for partial, not well defined blocks at G_1 and G_2 , to account for various physiological states.

Then there are two categories of non-cycling cells. One is called the G_1 population and it remains blocked in G_1 , and the other is called the G_2 population and stays blocked in G_2 . These two populations, G_1 and G_2 , are capable of moving into the proliferative pool upon specific stimulation. Thus the G_2 population constitutes a pool of potential new cells that are quickly available and useful in repair functions (Gelfant, 1976).

Now using radioisotope techniques it has been found that a small but significant increase in rate of labelling during the S phase could be detected at midnight—but not at other times (Kahn, Weinstein & Frost, 1968). Thus, for the time period of our study—9 a.m. to 3 p.m. there was no significant change in the number of cells entering the S phase of the cycle.

Therefore the increased number of mitoses we found with diurnal variation at 3 p.m. could not come from the G_1 population, nor from the cycling cell population partial block at G_1 , since a release of cells from the G_1 block would show an increased labelling in the S phase. It is likely then that these mitotic cells came from the G_2 population by a release of its G_2 block, or from the cycling cell population, by a release of its proposed partial block at G_2 , or from both sources. Thus diurnal interaction with the controls of the block at G_2 of the G_2 population, and or, of the cycling cell population, could account for the afternoon rise in count of mitotic figures in our study.

To explain our finding of a greater mitotic cell count at 3 p.m. in the uninvolved skin of psoriatics compared to normal skin, we may postulate a larger number of cells blocked at G_2 . It may be that this is due to a larger G_2 population in psoriatic epidermis.

In this connection it is of interest that in the plaque of psoriasis proper there is no diurnal variation in mitotic count (Milstein & Cornell, 1973). Hence it may be that in psoriasis there is a larger G_2 population as per above, and that the controls of $G_2 \rightarrow M$ block of the G_2 population or cycling cell population or both, are unstable. When these unstable controls break down, there is no further diurnal variation but a higher mitotic rate, cell proliferation and scaling. All this is in accord with previous reports suggesting a role for a G_2 population in psoriasis (Goodwin *et al.*, 1974; Gelfant, 1976).

On the premise that induction of the psoriatic process involves at least in part a breakdown of the normal controls blocking the $G_2 \rightarrow M$ transition of G_2 population, the colchicine studies discussed before are of interest. They may indicate a specific inhibition of the $G_2 \rightarrow M$ shift of the G_2 population by colchicine.

Such a specific action might be of considerable therapeutic value in psoriasis. Colchicine itself is quite toxic (Gaylarde & Sakarny, 1976). Search for an analogue or derivative of colchicine with less toxicity while retaining its $G_2 \rightarrow M$ block action on the G_2 population would seem a likely area for investigation.

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REFERENCES

- DIXON, W.J. (1975) In: *Biomedical Computer Programs*, pp. 711-760. University of California Press, Los Angeles.
- EVENSEN, A. (1963) Kinetics of epidermal cell proliferation in experimental skin carcinogenesis. *Bulletin of the World Health Organization*, **28**, 513.
- FISHER, L.B. (1968) Determination of the normal rate and duration of mitoses in human epidermis. *British Journal of Dermatology*, **80**, 24.
- FISHER, L.B. & WELLS, G.C. (1968) The mitotic rate and duration in lesions of psoriasis and ichthyosis. *British Journal of Dermatology*, **80**, 235.
- GAYLARDE, P.M. & SAKARNY, I. (1976) Colchicine therapy for psoriasis. *Archives of Dermatology*, **112**, 556.
- GELFANT, S. (1976) The cell cycle in psoriasis: a reappraisal. *British Journal of Dermatology*, **95**, 577.
- GOODWIN, P., HAMILTON, S. & FRY, L. (1974) The cell cycle in psoriasis. *British Journal of Dermatology*, **90**, 517.
- HOOPER, C.E. (1961) Use of colchicine for the measurement of mitotic rate in the intestinal epithelium. *American Journal of Anatomy*, **108**, 231.
- KAHN, G., WEINSTEIN, G.D. & FROST, P. (1968) Kinetics of human epidermal cell proliferation, diurnal variation. *Journal of Investigative Dermatology*, **50**, 459.
- MILSTEIN, H.G. & CORNELL, R.C. (1973) Diurnal mitotic studies of psoriatic epidermis. *Journal of Investigative Dermatology*, **61**, 180.
- ROWE, L. & DIXON, W.J. (1972) Clustering and control of mitotic activity in human epidermis. *Journal of Investigative Dermatology*, **58**, 16.
- WEINSTEIN, G.D. & FROST, P. (1968) Abnormal cell proliferation in psoriasis. *Journal of Investigative Dermatology*, **50**, 254.

The relationship between cyclic adenosine 3', 5' - monophosphate and biochemical events in rat skin after the induction of epidermal hyperplasia using hexadecane

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SUMMARY

Sequential changes in skin metabolism have been studied in a model system of epidermal hyperplasia and hyperkeratinization induced by the application of n-hexadecane to shaved rat skin. The epidermal accumulation of glycogen typical of the hyperplastic response has been correlated with an increase in glycogenesis and a decrease in glycogenolysis. DNA synthesis was increased by 6 h after the start of hexadecane treatment and reached a maximum after one day. The concentration of skin cyclic AMP fell immediately after hexadecane application and subsequently rose to give a prolonged increase. Use of the combined topical application of hexadecane and the anti-inflammatory drugs triamcinolone acetonide, hydrocortisone and indomethacin showed that the hexadecane-induced changes in DNA synthesis and glycogen metabolism were linked to the initial fall in cyclic AMP concentration. The significance of the biphasic change in cyclic AMP levels is discussed as a possible system of control for the development and maintenance of hyperplasia.

The epidermis is a continuously proliferating and differentiating tissue and, as such, places considerable demands upon many biochemical processes. The mechanism and control of these processes in skin are poorly understood. Some insight has been provided by the study of hyperproliferative conditions following experimentally produced skin injuries, e.g. the topical application of chemicals (Skjaeggsted, 1964; Baird *et al.*, 1971; Raick *et al.*, 1972; Grimm & Marks, 1974; Prottey & Hartop, 1973), removal of the stratum corneum by repeated application of cellophane tape (Christophers & Braun-Falco, 1970; Lobitz *et al.*, 1962) and exposure of the skin to ultraviolet light (Blum & Soffen, 1961; Eaglstein & Weinstein, 1975; Ohkawara *et al.*, 1972). Information has also been derived from a study of skin tumours (Goltz *et al.*, 1958; Ryan & Heidrick, 1974), the healing margins of skin wounds (Bilski & Godlewski, 1965; Im & Hoopes, 1970a; 1970b) and the epidermis of psoriatic lesions (Voorhees & Duell, 1975; Halprin *et al.*, 1975).

The induction of epidermal hyperplasia by the topical application of alkanes possessing fourteen to eighteen carbon atoms is well known (Brooks & Baumann, 1956; Bujard *et al.*, 1953; Hoekstra &

Phillips, 1963). The ^{16}C alkane n-hexadecane has been used in the study of skin hyperplasia (Ross-miller & Hoekstra, 1966) and to monitor the effects of drugs on the hyperplastic response (Weirich & Longauer, 1973; Weirich *et al.*, 1975). In the work described here the application of n-hexadecane to shaved rat skin has been used to investigate the chronology of biochemical changes occurring during the development of epidermal hyperplasia.

The effects of the anti-inflammatory drugs triamcinolone acetonide, hydrocortisone and indomethacin on hexadecane-induced hyperplasia have been used to demonstrate the relationship between these biochemical changes and the induction and development of the hyperplastic state.

MATERIALS AND METHODS

Materials

Male albino Wistar rats (200–230 g), CFHB strain, were obtained from Anglia Laboratory Animals, Alconbury, Huntingdon, U.K. All chemicals used were of analytical quality where possible. n-Hexadecane was obtained from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K. 1-Anilino naphthalene-8-sulphonic acid (Mg salt) was supplied by B.D.H. Chemicals Ltd, Poole, Dorset, U.K. Sodium pentobarbitone (Nembutal) was purchased from Abbott Laboratories Ltd, Queenborough, Kent, U.K. Arcton 12 (dichloro-difluoro-methane) spray was obtained from I.C.I. Ltd, Runcorn, Cheshire, U.K. DNA (Na salt) from calf thymus was supplied by Koch-Light Laboratories Ltd, Colnbrook, Bucks, U.K. Crystamycin and betamethasone were obtained from Glaxo Laboratories Ltd., Greenford, Middlesex, U.K. Triamcinolone acetonide was from E.R.Squibb and Sons Ltd., Liverpool, U.K. Hydrocortisone was purchased from Steraloids Ltd, Croydon, Surrey, U.K. Indomethacin was supplied by Merck, Sharp & Dohme Ltd., Hoddesdon, Herts, U.K. Phenylbutazone was obtained from Geigy Pharmaceuticals, Macclesfield, Cheshire, U.K. Aspirin was from CIBA Laboratories, Horsham, Sussex, U.K. Deoxycorticosterone, amyloglucosidase (E.C. 3.2.1.3), glycogen (type III—rabbit liver), Trizma-HCl, Trizma-Base and EDTA were obtained from Sigma Chemical Corporation, London, U.K. All other biochemicals and enzymes were purchased from The Boehringer Corporation (London) Ltd, Lewes, East Sussex, U.K. $[\text{U-}^{14}\text{C}]$ Glucose and $[\text{methyl-}^3\text{H}]$ thymidine were supplied by The Radiochemical Centre, Amersham, Bucks, U.K.

METHODS

Application of hexadecane

Hair was shaved from a 6×4 cm area of ventral skin of the rat without anaesthesia, using an Oster small animal clipper (Brookwick, Ward & Co., Ltd, London W6, U.K.) with a size 40 cutting head. The area used was from a point just above the penis to the xiphisternum. Animals were always shaved on the afternoon of the day preceding the first application of hexadecane.

Hexadecane (0.15 ml, 116 mg) was spread over the shaved area during a period of 20–30 s using a 0.2 ml pipette. The first application was at time 0 on day 0 of treatment. This was followed by two further applications at 4 hourly intervals on day 0, and three similar applications on day 1. Skin sampled 2 h after the start of treatment was taken 2 h after the first application, skin sampled at 6 h was taken 2 h after the second application and skin sampled at 10 h was taken 2 h after the third application. Skin sampled after 1 day of treatment was removed 24 h after the initial application and had received three applications of hexadecane. Similarly, skin treated for 2 days had received six applications and was sampled 48 h after the initial application. Skin samples taken 3–6 days after the start of treatment had also received a total of six applications of hexadecane.

After treatment the rats were housed individually with food and water *ad libitum*.

Topical application of drugs

Drugs were dissolved, at the desired concentration, in redistilled ethyl acetate. The solution (0.2 ml) was spread quickly over the whole area of shaved rat skin, and allowed to dry completely (5 s) before each application of hexadecane. Controls were carried out by application of the solvent (0.2 ml) in the same way.

The total quantities of drugs applied by the time of sampling were as follows:

Drug conc.	Time of sampling	Conc. applied ($\mu\text{g}/\text{cm}^2$)
1 mg/ml	6 h	16.7
	1 day	25.0
	2 days	50.0
0.1 mg/ml	6 h	1.67
	1 day	2.50
	2 days	5.00

Anaesthesia

Rats were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (60 mg/kg).

Preparation of skin samples

In all cases, except for the assay of cyclic AMP, skin samples were removed from the anaesthetized animal, blotted on filter paper and any subcutaneous fat and muscle quickly removed. The samples were then immersed in the appropriate buffered medium at 4°C for subsequent enzyme assay or incubation *in vitro*, or frozen in liquid nitrogen. For the assay of cyclic AMP a square of skin was dissected on three sides, the resulting flap of skin was lifted and instantly frozen using an Arcton 12 spray. The frozen skin was then removed from the animal and stored in liquid nitrogen. Skin powder was prepared by grinding skin pieces under liquid nitrogen for 90 min in a Spex Freezer Mill (Glen Creston, Stanmore, Middlesex, U.K.) at maximum speed.

Estimation of total skin glycogen

Skin powder (0.1–0.2 g) was digested in 1 M KOH (2 ml) by heating in a boiling water bath for 30 min. The solution, after cooling, was adjusted to pH 4 with 2 M HCl and diluted to 3.5 ml with water. After filtration an aliquot of the filtrate (2 ml) was mixed with 0.1 M sodium acetate buffer, pH 4.8 (2.5 ml) containing amyloglucosidase (1 mg/ml). The mixture was incubated at 50°C for 1 h followed by centrifugation. An aliquot (0.5 ml) of the supernatant was then used for the estimation of glucose as described below.

Estimation of glucose

Glucose was estimated by following the reduction of NADP⁺ at 25°C and 340 nm in a final volume of 3.11 ml, containing: 60 mM Tris-HCl pH 7.4, 12 mM MgCl₂, 0.4 mM NADP⁺, 0.32 mM ATP, 3.5 units of glucose-6-phosphate dehydrogenase and 2.8 units of hexokinase. The reaction was started by the addition of hexokinase and the change in optical density was recorded.

Estimation of DNA

DNA was extracted from skin powder (0.1–0.2 g) after removal of lipid by a modification of the

procedure of Schneider (1945) utilizing an ethanolic solution of sodium acetate after the initial trichloroacetic acid extractions (Marko & Butler, 1951). The DNA was finally extracted with 2×3 ml 1 M perchloric acid. A portion (1 ml) of this extract was then used for the estimation of DNA by the diphenylamine method of Burton (1956).

Estimation of cyclic AMP (adenosine-3', 5'-cyclic monophosphate)

Cyclic AMP was extracted from skin powder (0.5 g) using 0.5 M perchloric acid in 25% (w/v) ethanol (4.5 ml) at 4°C for 60 min, with repeated mixing on a vortex mixer. After centrifugation at 26,000 g for 15 min, an aliquot of the supernatant (4 ml) was neutralized with cold 30% (w/v) KOH, 0.4 M KCl and left in ice for 30 min to achieve complete precipitation of potassium perchlorate. The precipitate was removed by centrifugation and the decanted supernatant, containing cyclic AMP, was partially purified by the addition of 0.2 M Ba(OH)₂ (0.2 ml) and a neutralizing volume of 0.2 M ZnSO₄ (Krishna *et al.*, 1968) using phenolphthalein as an indicator. After precipitation of the BaSO₄ for 10 min in ice the mixture was centrifuged for 15 min at 26,000 g and portions (0.4 ml) of the supernatant dried under vacuum over P₂O₅. These were stored at -20°C until required for assay. Cyclic AMP recovery was estimated by including 0.1 µCi of cyclic [³H] AMP in the extraction medium. Recovery was in the range 84–95%. The dried sample was reconstituted with assay buffer (50 mM Tris-HCl, pH 7.4, 8 mM theophylline, 6 mM 2-mercaptoethanol) (1 ml). Aliquots (50 µl) of this solution were assayed for cyclic AMP using the saturation assay method of Brown *et al.* (1971) and Albano *et al.* (1974). The specificity of the assay was determined by the removal of cyclic AMP using cyclic nucleotide phosphodiesterase (Voorhees *et al.*, 1972).

Enzyme assays

Glycogen synthetase (UDP-glucose: α-1,4-glucan, α-4-glucosyltransferase (E.C. 2.4.1.11) was assayed by modification of the methods of Halprin & Ohkawara (1966a) and Passonneau & Rottenberg (1973).

Skin was dissected into ice-cold buffered medium containing 50 mM Tris-HCl pH 7.8, 0.5 mM EDTA, 3 mM MgCl₂, 5 mM dithiothreitol and 25 mM NaF. After chopping the tissue finely with scissors, portions (1 g) were homogenized in the same medium (6 ml) using a Polytron homogenizer (type P.T.10—Northern Media Supply, Hull, U.K.) at maximum speed for four periods of 30 s each, allowing the probe to cool between homogenization periods. After centrifugation of the homogenate at 26,000 g for 30 min the supernatant was filtered and the filtrate was used for the spectrophotometric assay. The assay medium contained, in a final volume of 3.11 ml; 50 mM Tris-HCl pH 8.0, 0.5 mM EDTA, 3 mM MgCl₂, 1.5 mg glycogen, 0.64 mM phosphoenolpyruvate, 1.2 mM UDP-glucose, 0.23 mM NADH, 9 units of lactate dehydrogenase and 3 units of pyruvate kinase. A similar assay medium containing no UDP-glucose was prepared as a blank. The reaction was started by the addition of filtered supernatant (0.1 ml) to both test and blank cuvettes and the rate of oxidation of NADH followed at 340 nm and 25°C. This rate was a measure of glycogen synthetase I (G6P independent). Glucose-6-phosphate (20 µmol) was added and the new rate measured. This was a measure of total glycogen synthetase activity (I + D).

Phosphorylase (α-1,4-glucan-orthophosphate glucosyl-transferase—EC.2.4.1.1.) was assayed by a modification of the method of Halprin & Ohkawara (1966a). A supernatant was prepared in the same way as described for the assay of glycogen synthetase except that homogenization was carried out in a buffered medium containing 50 mM Tris-HCl pH 7.2, 0.5 mM EDTA and 3 mM MgCl₂. The assay medium contained, in a final volume of 3.11 ml: 50 mM Tris-HCl pH 7.2, 0.5 mM EDTA,

3 mM MgCl_2 , 3 mg glycogen, 0.4 mM NADP^+ , 16 mM NaH_2PO_4 , 1.3 units of glucose-6-phosphate dehydrogenase and 1 unit of phosphoglucumutase. A similar medium was prepared as a blank containing no inorganic phosphate. The reaction was started by the addition of filtered supernatant (0.2 ml) to both test and blank cuvettes and the reduction of NADP^+ followed at 340 nm and 25°C. This initial rate was a measure of phosphorylase 'a' activity (AMP independent). After the addition of AMP (3 μmol) the new rate was measured which was a measure of total phosphorylase activity ('a' + 'b').

All enzyme activities were calculated as units/g skin (wet weight). 1 unit of enzyme activity is equivalent to 1 μmol of product formed per min at 25°C.

Incorporation studies in vitro

For the measurement of glucose incorporation into skin glycogen the incubation medium contained Krebs-Ringer bicarbonate buffer pH 7.2 containing penicillin (50 units/ml) and streptomycin (50 $\mu\text{g/ml}$) (Crystamycin), 5 mM $[\text{U}-^{14}\text{C}]\text{glucose}$ (12.5 μCi) and chopped skin pieces (1 g) in a total volume of 5 ml. The skin was incubated at 37°C for 1 h in an atmosphere of $\text{O}_2 + \text{CO}_2$ (95:5), followed by rinsing and blotting of the skin and isolation of glycogen. Radio-labelled glycogen was isolated and hydrolysed using amyloglucosidase as described by Peters & White (1976). Radioactivity was measured in scintillator fluid (5 ml) prepared according to Bray (1960).

Incorporation studies in vivo

The specific radioactivity of skin glycogen was measured 1 h after the intravenous injection of $[\text{U}-^{14}\text{C}]\text{glucose}$ (0.2 ml containing 10 μCi , 2 mCi/mmol). Skin samples were removed and frozen in liquid nitrogen followed by isolation and estimation of glycogen as described previously.

The specific radioactivity of skin DNA was measured 2 h after the intravenous injection of [methyl- ^3H]thymidine (0.2 ml containing 40 μCi , 5 Ci/mmol). DNA was isolated from powdered skin (0.5 g) by the method of Morley & Kingdon (1972) and estimated using the diphenylamine method of Burton (1956).

RESULTS AND DISCUSSION

The topical application of hexadecane to rat skin caused erythema followed by thickening and stiffening of the skin, the development of punctate cutaneous haemorrhage, generalized fissuring, cracking, and scaling of the keratin layer. Desquamation of large horny flakes from the skin surface was evident 3–4 days after the start of treatment and by 5 days the complete loss of the superficial horny layer revealed a skin surface of virtually normal appearance. Hoekstra & Philips (1963), Cowan & Mann (1971) and Christophers & Braun-Falco (1970) have reported a similar response following the topical application of hexadecane to guinea-pig skin. These effects appear to be typical of the hyperplastic hyperkeratinized condition in epidermis and have been observed after the topical application of strong soap solutions (Prottey & Hartop, 1973) and in the epidermis of psoriatic lesions (Baker & Wilkinson, 1972). The superficial changes in the skin were accompanied by an increase in the number of cell layers in the epidermis from 2 to about 10 three days after the start of treatment, by the appearance of acanthosis, acantholysis and parakeratosis, and by an initial transitory polymorphonuclear leukocyte invasion of the dermis. The histological and ultrastructural changes in the epidermis of guinea-pigs after topical application of hexadecane have been described in detail by Cowan & Mann (1971).

The gross changes produced by hexadecane application appear to be confined mainly to the epidermis but the results that follow have been obtained using whole skin samples (epidermis plus dermis). This produces some problems in the interpretation of results due to the heterogeneity of the

tissue; however, in the study of skin biochemistry there is always a choice between the use of whole intact skin containing a considerable but constant amount of relatively inert dermal tissue or the use of epidermal specimens damaged by chemical treatment or containing an unknown and variable amount of dermal contamination (Halprin, 1973).

The induction of skin hyperplasia was reflected by the change in the mass of full thickness skin measured as mg/cm^2 , which, although unchanged at 2 h and 6 h after the initial application of hexadecane, increased to 215% of control after 2 days (Fig. 1). Control levels were re-established by day 6.

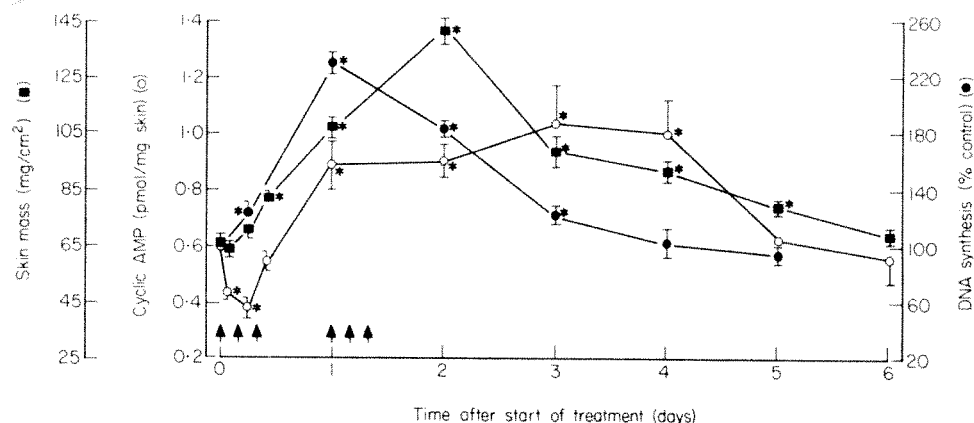


FIGURE 1. Skin mass, DNA synthesis and cyclic AMP concentration in skin after topical treatment with hexadecane. Skin mass was measured as mg/cm^2 of a 6.25 cm^2 area of ventral skin frozen in liquid nitrogen after removal of underlying fat and muscle. Each point is the mean result from 5 animals $\pm$ s.e.m.

DNA synthesis was measured as the specific radioactivity of DNA isolated from skin 2 h after the administration *in vivo* of a pulse of [methyl- ^3H] thymidine. Each point is the mean result from 4 animals $\pm$ s.e.m. The specific radioactivity of DNA from control skin (zero time) was $25.7 \text{ d.p.m.}/\mu\text{g}$ DNA.

Cyclic AMP was measured as described in the Methods section and expressed as pmol/mg skin (wet weight). Each point is the mean result from 10 animals $\pm$ s.e.m. Points marked * are significantly different from the mean at zero time ($P < 0.05$, Student's *t*-test).

The arrows indicate single applications of hexadecane.

Glycogen metabolism

An increase in the glycogen content of epidermis is a characteristic of the hyperplastic state and has been observed in the margins of healing skin wounds (Bilski & Godlewski, 1965; Williams, 1972), after exposure of skin to ultraviolet light (Ohkawara *et al.*, 1972; Lobitz *et al.*, 1962), after stripping of the stratum corneum with cellophane tape (Lobitz *et al.*, 1962), after treatment of the skin with carcinogens (Argyris, 1952), and in hyperproliferative skin diseases such as psoriasis (Halprin & Ohkawara, 1966b; Halprin *et al.*, 1973). Hexadecane-induced hyperplasia also produced an increase in skin glycogen which became significantly different from control two days after the start of hexadecane treatment, reached a maximum on the 4th day and was then maintained until the 6th day (Fig. 2).

Measurement of the rates of incorporation of [$\text{U-}^{14}\text{C}$] glucose into skin glycogen in both the *in vitro* and *in vivo* states (Fig. 3) showed that the synthesis of glycogen from glucose was stimulated by the induction of epidermal hyperplasia. This was corroborated by measurement of the activities of the rate-limiting enzymes controlling glycogenesis and glycogenolysis, namely glycogen synthetase and phosphorylase.

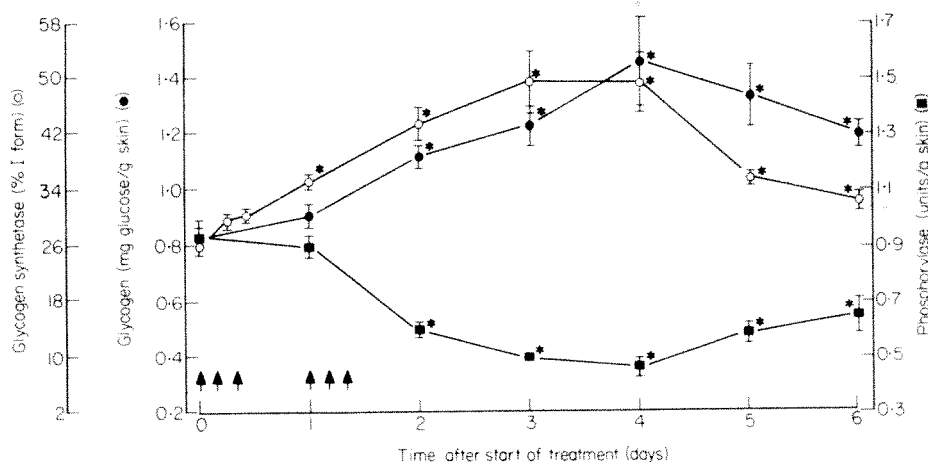


FIGURE 2. The concentration of glycogen and the activities of glycogen synthetase and phosphorylase in skin after topical treatment with hexadecane. Glycogen was measured as glucose equivalents and is expressed as mg glucose/g skin (wet weight). The assay is described in the Methods section.

The activity of the 'I' form of glycogen synthetase is expressed as a percent of the total enzyme activity. Total glycogen synthetase activity in control skin (zero time) was 0.73 units/g skin (wet weight). Total phosphorylase activity is expressed as units/g skin (wet weight). Details of the enzyme assays are given in the Methods section. Each point is the mean result from 5 animals $\pm$ s.e.m. Points marked * are significantly different from the mean at zero time ($P < 0.05$, Students 't'-test). The arrows indicate single applications of hexadecane.

It was found that phosphorylase was almost entirely in the AMP dependent 'b' form in rat skin, the 'a' form of the enzyme comprising only 5% of the total in control skin. Comparison of the activities after hexadecane treatment showed no significant change in the proportion of phosphorylase 'a' but total phosphorylase activity had decreased significantly by day 2 and had reached 51% of control by day 4 (Fig. 2).

No significant differences were seen in the total activity of glycogen synthetase in skin after hexadecane treatment, but a marked effect was demonstrated in the relative proportions of the glucose-6-phosphate independent (I) form of the enzyme and the glucose-6-phosphate dependent (D) form. In control skin the activity of the 'I' form was 26% of the total activity (0.73 units/g skin). This had risen significantly by 6 h after the start of treatment and increased in a linear fashion to a maximum of 49.3% on days 3 and 4 after which there was a gradual return towards the control level (Fig. 2).

The relationship between glycogen deposition, glucose incorporation into glycogen and the activities of glycogen synthetase and phosphorylase has been studied in psoriatic skin (Halprin & Ohkawara, 1966b; Halprin *et al.*, 1973) but the results of this work did not explain the mechanisms of glycogen deposition in hyperplastic epidermis. The pattern seen in rat skin after hexadecane treatment is more closely paralleled by the effects of ultraviolet light on mini-pig skin (Ohkawara *et al.*, 1972). In this case a 2-3-fold increase in the glycogen content of epidermis commencing 17 h after irradiation was preceded by a massive 9-10-fold increase in the activity of glycogen synthetase 'I', and a decrease in total phosphorylase activity. These changes were not reflected by the rate of glucose incorporation *in vitro* into epidermal glycogen. The authors attributed this to negative feedback related to the elevated tissue level of glycogen, but their data do not fully substantiate this argument.

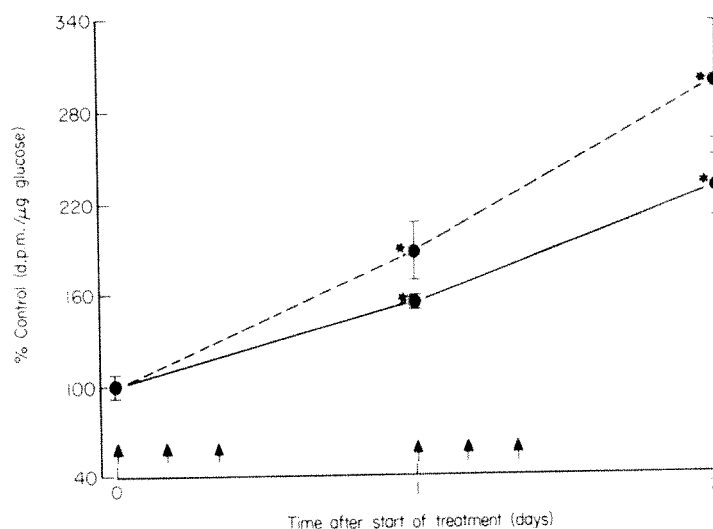


FIGURE 3. Incorporation *in vitro* (—) and *in vivo* (---) of [U- ^{14} C] glucose into the glycogen of skin after topical treatment with hexadecane. Details of the incubation and of the isolation and determination of the glucose residues of glycogen are given in the Methods section.

Each point is the mean result from 4 animals $\pm$ s.e.m. Points marked * are significantly different from the mean at zero time ($P < 0.05$, Student's t -test). The specific radioactivities of the glucose residues of glycogen from control skin (zero time) after [U- ^{14} C] glucose incorporation *in vitro* and *in vivo* were 164.6 and 0.82 d.p.m./μg glucose respectively.

The arrows indicate single applications of hexadecane.

DNA synthesis

DNA synthesis was measured by calculation of the specific radioactivity of DNA isolated from skin after the administration of an intravenous pulse of [methyl- ^{3} H]thymidine. The rate of incorporation of thymidine expressed in this way is proportional to the number of cells engaged in DNA synthesis during the period of exposure to thymidine. As DNA duplication is a prerequisite for mitotic division it can be assumed that this measurement is directly related to the mitotic index which indicates the relative number of cells undergoing mitosis at a given moment.

In skin after hexadecane treatment the rate of thymidine incorporation *in vivo* into skin DNA was elevated significantly 6 h after the initial treatment, reached a maximum at 1 day followed by a gradual return to normal control levels by day 4 (Fig. 1).

Prottey & Hartop (1973) have reported changes in thymidine incorporation after the induction of hyperplasia in rat skin using strong soap solutions which are almost identical to those observed after hexadecane treatment. However, other reports of changes in DNA synthesis related to epidermal hyperplasia, such as those seen after ultraviolet irradiation (Epstein *et al.*, 1970; Krämer *et al.*, 1974) and the application of the tumour promoting phorbol diesters (Baird *et al.*, 1971; Raick *et al.*, 1972; Kreig, *et al.*, 1974), are complicated by the effects of the inducer on DNA structure itself (Marmur & Grossman, 1961) or to a high degree of cytotoxicity (Raick, 1973) both of which tend to produce an early depression in DNA synthesis.

Cyclic AMP

Cyclic AMP has been closely linked with the control of cell proliferation (Posternak, 1974) and glycogen metabolism (Soderling & Park, 1974). The decreased concentration of cyclic AMP in psoriatic

epidermis described by Voorhees (Voorhees *et al.*, 1975; Voorhees & Duell, 1975) suggested that the epidermal hyperproliferation and glycogen deposition associated with this disease were attributable to changes in cyclic AMP. However, these findings have been disputed by Halprin *et al.* (1975) and Harkon n *et al.* (1974) who demonstrated an unchanged or elevated concentration of cyclic AMP in epidermis from psoriatic lesions. Measurement of cyclic AMP concentrations in a tissue exhibiting an established hyperplastic condition, such as psoriatic epidermis, does not indicate the possible relationship between cyclic AMP and the development of that hyperplasia. Study of cyclic AMP concentration in skin during the development of hexadecane-induced hyperplasia has shown that this relationship is complex. An initial decrease in the concentration of the nucleotide was seen immediately after the start of hexadecane treatment; however, this was transitory and was followed by a rise in concentration into a broad peak terminating 5 days after the start of treatment (Fig. 1).

Treatment of mouse skin with the hyperplasia-producing tumour promotor tetradecanoyl-phorbol acetate (TPA) produced a similar response (Grimm & Marks, 1974). In this case there was an initial elevation of cyclic AMP 1–2 h after treatment followed by a decrease below control levels at 12–18 h and a sustained increase at 30 h. The initial increase in cyclic AMP using TPA, which was not evident using hexadecane, has not been explained but could presumably be related to the greater cytotoxicity of the phorbol ester which is also thought to be responsible for the rapid decrease in DNA synthesis at this time (Raick *et al.*, 1972).

The control of hexadecane-induced hyperplasia

The earliest change observed in skin after the application of hexadecane was in the concentration of cyclic AMP. The initial events up to 6 h suggest that the increase in both DNA synthesis and glycogenesis could be ascribed to a previous and rapid decrease in the concentration of cyclic AMP. This is consistent with the theory that cell proliferation and glycogen accumulation in hyperplastic epidermis are controlled by changes in the concentration of cyclic AMP (Voorhees & Duell, 1975). After the 6 h point the picture becomes more complicated as the cyclic AMP concentration begins to rise. This presents a situation of increased cell proliferation during a period of elevated cyclic AMP concentration. This type of relationship has been observed previously in regenerating liver (MacManus *et al.*, 1972), in rapidly growing tumours (Thomas *et al.*, 1973) and in epidermis after the application of carcinogen (Verma & Murray, 1974) even though the hyperplasia associated rise in epidermal glycogen contradicts the normally accepted theory of the cyclic AMP control of glycogen metabolism (Soderling & Park, 1974).

The combined topical application of hexadecane and anti-inflammatory drugs has been used in an attempt to clarify any connection between cyclic AMP changes and the induction of hyperplasia.

The combined topical application of triamcinolone acetonide (0.1 mg/ml) and hexadecane prevented the initial depression of cyclic AMP seen 6 h after the application of hexadecane plus vehicle (Table 1), completely inhibited the elevation of DNA synthesis at 1 day (Table 2), reduced the rise in the active form of glycogen synthetase at 2 days from 167% of control to 127% of control (Table 3) and inhibited the increase in skin mass by 72% at day 1 and 57% at day 2 (Table 4) and the development of hyperplasia. Neither hydrocortisone (0.1 mg/ml) nor indomethacin (1 mg/ml) affected the initial drop in cyclic AMP (Table 1) or the changes in glycogen synthetase (Table 3). Indomethacin had no effect on the increased level of DNA synthesis which was slightly reduced by hydrocortisone (Table 2). While both these drugs affected the increase in skin mass (Table 4) and the development of hyperplasia and hyperkeratinization their inhibitory effect was small compared with that of triamcinolone acetonide. All three drugs completely abolished the rise in cyclic AMP seen at 1 and 2 days after the start of hexadecane treatment.

TABLE 1. The concentration of cyclic AMP (per cent control) in skin after topical hexadecane and drug treatment

Time after first application (h)		Ethyl acetate (vehicle)	Indomethacin (1.0 mg/ml)	Hydrocortisone (0.1 mg/ml)	Triamcinolone acetoneide (0.1 mg/ml)
Hexadecane	Drug/vehicle				
	6	100 ± 8.50	94.9 ± 9.60	105.1 ± 8.95	103.4 ± 7.90
6	6	64.4 ± 7.63*	61.7 ± 4.17*	72.4 ± 4.75*	94.2 ± 4.54
24	24	150.8 ± 15.05*	98.6 ± 7.93	111.5 ± 14.7	95.1 ± 12.4
48	48	152.5 ± 10.15*	108.8 ± 6.00	114.1 ± 11.10	101.0 ± 12.10

Each result is the mean of ten animals ± s.e.m.

* Significantly different from ethyl acetate or drug alone ($P < 0.05$, Student's 't'-test).

Details of the assay are given in the Methods section.

Hexadecane was applied as shown in the figures.

These results are strongly indicative of an association between the initial fall in cyclic AMP and the changes characteristic of the hyperplastic state.

It is of course possible that the initial decrease in cyclic AMP was due simply to the toxic effects of hexadecane and was unrelated to the following changes in DNA synthesis. This possibility has been suggested by Grimm & Marks (1974) who showed a similar early decrease in epidermal cyclic AMP levels after the topical application of TPA. This could be correlated with a later increase in DNA synthesis (Krieg *et al.*, 1974) but they could not detect any decrease in cyclic AMP levels using the weaker tumour promotor phorbol dibenzoate which also stimulated DNA synthesis (Baird *et al.*, 1971; Krieg *et al.*, 1974). However, hexadecane does not produce tissue effects as severe as those

TABLE 2. Incorporation *in vivo* of [methyl-³H]-thymidine into the DNA of skin after topical hexadecane and drug treatment for one day

Treatment	Drug concentration (mg/ml)	d.p.m./μg DNA (% control)
Control (ethyl acetate)	—	100 ± 2.5*
Ethyl acetate + hexadecane	—	229.1 ± 8.0†
Triamcinolone acetoneide alone	0.1	54.9 ± 6.6*†
Triamcinolone acetoneide + hexadecane	0.1	103.0 ± 3.0*
Indomethacin alone	1.0	91.0 ± 8.5*
Indomethacin + hexadecane	1.0	200.3 ± 7.2†
Hydrocortisone alone	0.1	84.7 ± 0.9*†
Hydrocortisone + hexadecane	0.1	186.4 ± 8.2*†

* Significantly different from ethyl acetate + hexadecane ($P < 0.05$, Student's 't'-test).

† Significantly different from control ($P < 0.05$, Student's 't'-test).

Each result is the mean from five animals ± s.e.m.

Experimental procedures are described in the Methods section.

Hexadecane was applied 3 times daily as shown in the figures.

TABLE 3. The relative activity of glycogen synthetase I in skin after topical hexadecane and drug treatment for 2 days

Treatment	Drug concentration (mg/ml)	Synthetase I as % of total synthetase activity
Control (ethyl acetate)	—	28.8 ± 0.69*
Ethyl acetate + hexadecane	—	48.0 ± 2.40†
Triamcinolone acetonide + hexadecane	0.1	36.6 ± 1.11*†
Triamcinolone acetonide + hexadecane	1.0	30.2 ± 1.57*
Indomethacin + hexadecane	1.0	46.4 ± 0.98†
Hydrocortisone + hexadecane	0.1	46.6 ± 1.06†

* Significantly different from ethyl acetate + hexadecane ($P < 0.05$, Student's 't'-test).

† Significantly different from control ($P < 0.05$, Student's 't'-test).

Each result is the mean from five animals ± s.e.m.

The assay method is described in the Methods section.

Topical administration of the drugs alone produced no significant differences from control.

Hexadecane was applied as shown in the figures.

TABLE 4. Skin mass after topical hexadecane and drug treatment

Treatment	Drug concentration (mg/ml)	1 Day after start of treatment		2 Days after start of treatment	
		Skin mass (mg/cm ²)	% Inhibition of increase	Skin mass (mg/cm ²)	% Inhibition of increase
Control (no treatment)	—	65.7 ± 2.5	—	65.7 ± 2.5	—
Ethyl acetate + hexadecane	—	99.9 ± 1.6	—	145.1 ± 2.9	—
Triamcinolone acetonide + hexadecane	1.0	63.7 ± 1.5	100	78.6 ± 2.2	83.8
Triamcinolone acetonide + hexadecane	0.1	75.2 ± 2.6	72.2	99.7 ± 3.6	57.2
Hydrocortisone + hexadecane	1.0	80.2 ± 2.3	57.6	109.7 ± 5.1	44.6
Hydrocortisone + hexadecane	0.1	91.4 ± 2.9	24.9	123.1 ± 1.3	27.7
Indomethacin + hexadecane	1.0	93.4 ± 1.1	19.0	122.0 ± 4.3	29.1

Each result is the mean from five animals ± s.e.m.

Hexadecane was applied as shown in the figures.

seen after TPA treatment (Baird *et al.*, 1971; Raick, 1973) and more specifically does not cause the early transitory decrease in DNA synthesis (Raick *et al.*, 1972; Baird *et al.*, 1971) which has been attributed to the highly toxic effects of TPA but which is not evident after phorbol dibenzoate treatment (Baird *et al.*, 1971).

Assuming that development of hyperplasia and glycogen accumulation in the epidermis after hexadecane application were triggered by the initial drop in cyclic AMP, there must be some other explanation for the subsequent increase in the concentration of the nucleotide.

It has been suggested (Grimm & Marks, 1974) that this type of increase is related to the induction

of epidermal cell differentiation. This certainly agrees with the fact that the biosynthesis of histidine-rich protein, which can be taken as a marker of epidermal functional differentiation, is increased in TPA treated epidermis after the increase in DNA synthesis (Krieg *et al.*, 1974) and that sensitivity to chalone-containing skin extracts, which was lost after TPA treatment, was re-established at the same time (Krieg *et al.*, 1974). It has also been shown that the addition of dibutyryl cyclic AMP to epidermal cells in culture caused a significant increase in keratinization and the incorporation of amino acids into the granular layer of the epidermis (Delescluse *et al.*, 1976). These results support the theory which implicates cyclic GMP as the main controlling factor of cell division and proliferation and cyclic AMP as the regulator of epidermal cell function and differentiation (Voorhees & Duell, 1975).

As the increase in cyclic AMP after hexadecane treatment coincided with the appearance of erythema and polymorphonuclear leukocyte invasion of the dermis, it is also possible that it is connected with the inflammatory response produced by hexadecane. Cyclic AMP elevation may be induced by the release of prostaglandins which has been shown to parallel cutaneous inflammation (Goldyne, 1975; Greaves & McDonald-Gibson, 1973). Prostaglandins of the E type cause elevation of skin cyclic AMP (Kuehl, 1974; Goldyne, 1975; Adachi *et al.*, 1975; Aso *et al.*, 1975) and induce cyclic AMP formation in cultured fibroblasts (O'Neill & Hsie, 1975).

It is well known that prostaglandin biosynthesis is inhibited by indomethacin (Vane, 1971) and there is good evidence that hydrocortisone reduces prostaglandin release (Lewis & Piper, 1975). The fact that both these drugs and the potent anti-inflammatory agent triamcinolone acetonide all inhibit the hexadecane-induced increase in skin cyclic AMP concentration agrees with a theory of prostaglandin involvement.

REFERENCES

- ADACHI, K., YOSHIKAWA, K., HALPRIN, K.M. & LEVINE, V. (1975) Prostaglandins and cyclic AMP in epidermis. *British Journal of Dermatology*, **92**, 381.
- ALBANO, J.D.M., BARNES, G.D., MAUDSLEY, D.V., BROWN, B.L. & ETKINS, R.P. (1974) Factors affecting the saturation assay of cyclic AMP in biological systems. *Analytical Biochemistry*, **60**, 130.
- ARGYRIS, T.S. (1952) Glycogen in the epidermis of mice painted with methylcholanthrene. *Journal of the National Cancer Institute*, **12**, 1159.
- ASO, K., ORENBERG, E.K. & FARBER, E.M. (1975) Reduced epidermal cyclic AMP accumulation following prostaglandin stimulation: its possible role in the pathophysiology of psoriasis. *Journal of Investigative Dermatology*, **65**, 375.
- BAIRD, W.M., SEDGWICK, J.A. & BOUTWELL, R.K. (1971) Effects of phorbol and four diesters of phorbol on the incorporation of tritiated precursors into DNA, RNA and protein in mouse epidermis. *Cancer Research*, **31**, 1434.
- BAKER, H. & WILKINSON, D.S. (1972) Psoriasis. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J. Ebling), 2nd edn. Vol. 2, pp. 1192-1240. Blackwell Scientific Publications, Oxford.
- BILSKI, R. & GODLEWSKI, H.G. (1965) Histochemistry of glycogen, phosphorylases and UDPG-dehydrogenase in the epidermis of healing scald wound in guinea-pigs. *Folia Histochemica et Cytochemica*, **3**, 225.
- BLUM, H.F. & SOFFEN, G.A. (1961) Quantitative analysis of epidermal hyperplasia in mouse skin following single doses of ultraviolet light. *Journal of Cellular and Comparative Physiology*, **58**, 97.
- BRAY, G.A. (1960) A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analytical Biochemistry*, **1**, 279.
- BROOKS, S.C. & BAUMANN, C.A. (1956) Skin sterols. X. Studies with certain hyperplasia-inducing hydrocarbons. *Cancer Research*, **16**, 357.
- BROWN, B.L., ALBANO, J.D.M., ETKINS, R.P., SGHERZI, A.M. & TAMPION, W. (1971) A simple and sensitive saturation assay method for the measurement of adenosine 3':5'-cyclic monophosphate. *Biochemical Journal*, **121**, 561.
- BUJARD, E., JADASSOHN, W. & PAILLARD, R. (1953) Action de quelques hydrocarbures sur l'épiderme. *Dermatologica*, **106**, 161.

- BURTON, K. (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochemical Journal*, **62**, 315.
- CHRISTOPHERS, E. & BRAUN-FALCO, O. (1970) Mechanisms of parakeratosis. *British Journal of Dermatology*, **82**, 268.
- COWAN, M.A. & MANN, P.R. (1971) Histological and ultrastructural changes in experimental hyperplasia in the guinea-pig. *British Journal of Dermatology*, **84**, 353.
- DELESCLUSE, C., FUKUYAMA, K. & EPSTEIN, W.L. (1976) Dibutyryl cyclic AMP-induced differentiation of epidermal cells in tissue culture. *Journal of Investigative Dermatology*, **66**, 8.
- EAGLSTEIN, W.H. & WEINSTEIN, G.D. (1975) Prostaglandin and DNA synthesis in human skin: possible relationship to ultraviolet light effects. *Journal of Investigative Dermatology*, **64**, 386.
- EPSTEIN, J.H., FUKUYAMA, K. & FYE, K. (1970) Effects of ultraviolet radiation on the mitotic cycle and DNA, RNA and protein synthesis in mammalian epidermis *in vivo*. *Photochemistry and Photobiology*, **12**, 57.
- GOLDYNE, M.E. (1975) Prostaglandins and cutaneous inflammation. *Journal of Investigative Dermatology*, **64**, 377.
- GOLTZ, R.W., FUSARO, R.M. & JARVIS, J. (1958) Observations on glycogen in epithelial tumours. *Journal of Investigative Dermatology*, **31**, 331.
- GREAVES, M.W. & McDONALD-GIBSON, W. (1973) Effect of non-steroid anti-inflammatory and anti-pyretic drugs on prostaglandin biosynthesis by human skin. *Journal of Investigative Dermatology*, **61**, 127.
- GRIMM, W. & MARKS, F. (1974) Effect of tumour-promoting phorbol esters on the normal and the isoproterenol-elevated level of adenosine 3',5'-cyclic monophosphate in mouse epidermis *in vivo*. *Cancer Research*, **34**, 3128.
- HALPRIN, K.M. & OHKAWARA, A. (1966a) Glucose and glycogen metabolism in the human epidermis. *Journal of Investigative Dermatology*, **46**, 43.
- HALPRIN, K.M. & OHKAWARA, A. (1966b) Carbohydrate metabolism in psoriasis: an enzymatic study. *Journal of Investigative Dermatology*, **46**, 51.
- HALPRIN, K.M. (1973) Letter to the Editor. *Archives of Dermatology*, **108**, 725.
- HALPRIN, K.M., OHKAWARA, A. & LEVINE, V. (1973) Synthesis of glycogen in the psoriatic lesion. *Archives of Dermatology*, **107**, 706.
- HALPRIN, K.M., ADACHI, K., YOSHIKAWA, K., LEVINE, V., MUI, M.M. & HSIA, S.L. (1975) Cyclic AMP and psoriasis. *Journal of Investigative Dermatology*, **65**, 170.
- HÄRKÖNEN, M., HOPU-HAVU, V.K. & RAIJ, K. (1974) Cyclic adenosine monophosphate, adenylyl cyclase and cyclic nucleotide phosphodiesterase in psoriatic epidermis. *Acta dermato-venereologica*, **54**, 13.
- HOEKSTRA, W.G. & PHILLIPS, P.H. (1963) Effects of topically applied mineral oil fractions on the skin of the guinea-pigs. *Journal of Investigative Dermatology*, **40**, 79.
- IM, M.J.C. & HOOPES, J.E. (1970a) Enzyme activities in the repairing epithelium during wound healing. *Journal of Surgical Research*, **10**, 173.
- IM, M.J.C. & HOOPES, J.E. (1970b) Energy metabolism in healing skin wounds. *Journal of Surgical Research*, **10**, 459.
- KRÄMER, D.M., PATHAK, M.A., KORNHAUSER, A. & WISKEMAN, A. (1974) Effect of ultraviolet irradiation on biosynthesis of DNA in guinea-pig skin *in vivo*. *Journal of Investigative Dermatology*, **62**, 388.
- KRIEG, L., KÜHLMANN, I. & MARKS, F. (1974) Effect of tumor-promoting phorbol esters and of acetic acid on mechanisms controlling DNA synthesis and mitosis (chalones) and on the biosynthesis of histidine-rich protein in mouse epidermis. *Cancer Research*, **34**, 3135.
- KRISHNA, G., WEISS, B. & BRODIE, B.B. (1968) A simple, sensitive method for the assay of adenylyl cyclase. *Journal of Pharmacology and Experimental Therapeutics*, **163**, 379.
- KUEHL, F.A. (1974) Prostaglandins, cyclic nucleotides and cell function. *Prostaglandins*, **5**, 325.
- LEWIS, G.P. & PIPER, P.J. (1975) Inhibition of release of prostaglandins as an explanation of some of the actions of anti-inflammatory corticosteroids. *Nature*, **254**, 308.
- LOBITZ, W.C., BROPHY, D., LARNER, A.E. & DANIELS, F. (1962) Glycogen response in human epidermal basal cell. *Archives of Dermatology*, **86**, 207.
- MACMANUS, J.P., FRANKS, D.J., YOUNDALE, T. & BRACELAND, B.M. (1972) Increases in rat liver cyclic AMP concentrations prior to the initiation of DNA synthesis following partial hepatectomy or hormone infusion. *Biochemical and Biophysical Research Communications*, **49**, 1201.
- MARKO, A.M. & BUTLER, G.C. (1951) The isolation of sodium desoxyribonucleate with sodium dodecyl sulphate. *Journal of Biological Chemistry*, **191**, 165.
- MARMUR, J. & GROSSMAN, L. (1961) Ultraviolet light-induced linking of deoxyribonucleic acid strands and its reversal by photoreactivating enzyme. *Proceedings of National Academy of Science, U.S.A.*, **47**, 778.

- MORLEY, C.G.D. & KINGDON, H.S. (1972) Use of ^3H -thymidine for measurement of DNA synthesis in rat liver—a warning. *Analytical Biochemistry*, **45**, 298.
- OHKAWARA, A., HALPRIN, K.M. & LEVINE, V. (1972) Glycogen metabolism following ultraviolet irradiation. *Journal of Investigative Dermatology*, **59**, 264.
- O'NEILL, J.P. & HSIE, A.W. (1975) Prostaglandin E_1 induced changes in cyclic AMP levels in WI-38 human fibroblasts. Two different effects of cell density. *Biochimica et Biophysica Acta*, **404**, 243.
- PASSONNEAU, J.V. & ROTTENBERG, D.A. (1973) An assessment of methods for measurement of glycogen synthetase activity including a new direct one-step assay. *Analytical Biochemistry*, **51**, 528.
- PETERS, R.F. & WHITE, A.M. (1976) The existence of a glyconeogenic pathway in rat skin. *Biochemical Journal*, **156**, 465.
- POSTERNAK, T. (1974) Cyclic AMP and cyclic GMP. *Annual Reviews of Pharmacology*, **14**, 23.
- PROTTEY, C. & HARTOP, P.J. (1973) Changes in glycerolipid metabolism in rat epidermis following exaggerated washing with soap solutions. *Journal of Investigative Dermatology*, **61**, 168.
- RAICK, A.N. (1973) Ultrastructural, histological and biochemical alterations produced by 12-O tetradecanoyl-phorbol-13-acetate on mouse epidermis and their relevance to skin tumor promotion. *Cancer Research*, **33**, 269.
- RAICK, R.A., THUMM, K. & CHIVERS, B.R. (1972) Early effects of 12-O-tetradecanoyl-phorbol-13-acetate on the incorporation of tritiated precursor into DNA and the thickness of the interfollicular epidermis and their relation to tumor promotion in mouse skin. *Cancer Research*, **32**, 1562.
- ROSSMILLER, J.D. & HOEKSTRA, W.G. (1966) Hexadecane-induced hyperkeratinization of guinea-pig skin. IV. A comparison of free amino acid levels in normal and hyperkeratotic epidermis. *Journal of Investigative Dermatology*, **47**, 44.
- RYAN, W.L. & HEIDRICK, M.L. (1974) Role of cyclic nucleotides in cancer. In: *Advances in cyclic nucleotide research* (Ed. by P. Greengard and G.A. Robison), Vol. 4, pp. 81–116. Raven Press, New York.
- SCHNEIDER, W.C. (1945) Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid. *Journal of Biological Chemistry*, **161**, 293.
- SKJAEGGESTAD, Ø. (1964) Experimental epidermal hyperplasia in mice. Relation to carcinogenesis. *Acta Pathologica et Microbiologica Scandinavica*. Suppl. **169**, 1.
- SODERLING, T.R. & PARK, C.R. (1974) Recent advances in glycogen metabolism. In: *Advances in Cyclic Nucleotide Research* (Ed. by P. Greengard and G.A. Robison), Vol. 4, pp. 283–333. Raven Press, New York.
- THOMAS, E.W., MURAD, F., LOONEY, W.B. & MORRIS, H.P. (1973) Adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate: concentrations in Morris hepatomas of different growth rates. *Biochimica et Biophysica Acta*, **297**, 564.
- VANE, J.R. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature (New Biology)*, **231**, 232.
- VERMA, A.K. & MURRAY, A.W. (1974) The effect of benzo(a) pyrene on the basal and isoproterenol stimulated levels of cyclic adenosine 3',5'-monophosphate in mouse epidermis. *Cancer Research*, **34**, 3408.
- VOORHEES, J.J. & DUELL, E.A. (1975) Imbalanced cyclic AMP–cyclic GMP levels in psoriasis. In: *Advances in Cyclic Nucleotide Research* (Ed. by G.I. Drummond, P. Greengard and G.A. Robison), Vol. 5, pp. 735–758. Raven Press, New York.
- VOORHEES, J.J., DUELL, E.A., BASS, L.J., POWELL, J.A. & HARRELL, G.R. (1972) Decreased cyclic AMP in the epidermis of lesions of psoriasis. *Archives of Dermatology*, **105**, 695.
- VOORHEES, J.J., MARCELO, C.L. & DUELL, E.A. (1975) Cyclic AMP, cyclic GMP and glucocorticoids as potential metabolic regulators of epidermal proliferation and differentiation. *Journal of Investigative Dermatology*, **65**, 179.
- WEIRICH, E.G. & LONGAUER, J. (1973) Vergleichende Prüfung des epidermal antihyperplastischen Effektes von Externocorticoiden in Tierversuch. *Archiv für Dermatologie Forschung*, **248**, 179.
- WEIRICH, E.G., LONGAUER, J.K. & KIRKWOOD, A.H. (1975) Dermatopharmacology of salicylic acid. II. Epidermal antihyperplastic effect of salicylic acid in animals. *Dermatologica*, **151**, 321.
- WILLIAMS, J.P.G. (1972) Interrelation of epithelial glycogen, cell proliferation and cellular migration with cyclic adenosine monophosphate in epithelial wound healing. *Cell Differentiation*, **1**, 317.

A quantitative approach to epidermal wound healing: the effect of dexamethasone on regenerating epithelium

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SUMMARY

The effect of dexamethasone on the regeneration of new epithelium over the base of an excised suction blister has been studied over a period of 9 days. It was found that dexamethasone did not significantly affect the migration of epidermis over the blister base but it did significantly reduce the number of cells in DNA synthesis and post wounding acanthosis of wound edge and regenerating epithelium.

In order to develop drugs which influence wound healing it is necessary to have a technique which can measure its rate of progress. One of the greatest problems in this area is the complexity of the wound healing process: collagen formation, clot removal, tissue refashioning and re-epithelialization all play a role. We believe that a wound of the skin remains a wound as long as it is uncovered by mature functioning epidermis. We are therefore attempting to understand the process of re-epithelialization and have produced a model in which it can be easily measured. The model that we have chosen is that of the re-epithelializing area produced by suction blistering in the hairless hamster.

MATERIALS AND METHODS

Female hairless hamsters (Wright's, Essex) weighing between 90 and 120 g were used throughout the following experiments. Animals receiving dexamethasone treatment were given daily s.c. injections of dexamethasone (20 µg/0.2 ml saline/100 g body weight) commencing 1 week prior to blistering and during the healing period. All animals were lightly anaesthetized with intraperitoneal Nembutal (60 mg/kg) and blisters were produced on the dorso-lumbar region using suction cups of 3 mm internal diameter. The blisters formed easily using a negative pressure of 300 mmHg for 60–90 min (Fig. 1). After the blisters had formed, the blister roofs were carefully removed and the bases were lightly swabbed with 70% ethyl alcohol before covering the site with Blenderm surgical tape (3M Medical Products) (Fig. 2). The blister sites were allowed to re-epithelialize over a 9-day period.

Measurement of the rate of migration of epidermis over the blister base

Two distinct methods of evaluation were used:

(a) *Direct planimetry*. At the end of the observation period the animals were killed and the blister sites excised and fixed in 10% formalin. The whole preparation was then stained lightly in haematoxylin and eosin (stratum corneum staining pink-red, non-epithelialized areas staining blue) and examined in a Nikon stereo microscope. Every specimen was photographed using Kodak high speed Ektachrome film under standardized conditions. Each photograph was then projected onto a screen set at a standard distance (48 in) from the projecting lens. The image of the non re-epithelialized areas



FIGURE 1. Suction blisters produced by a negative pressure of 300 mmHg for 60–90 min in a female hairless hamster.

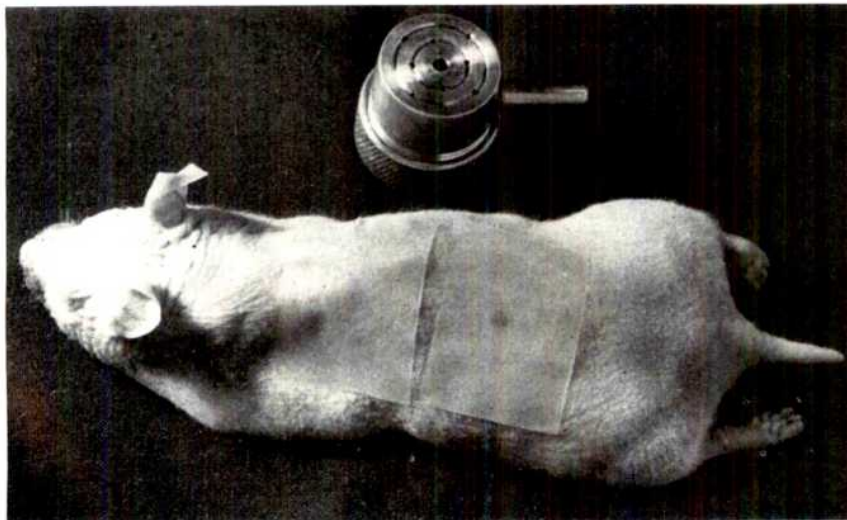


FIGURE 2. Excised suction blisters, covered with Blenderm surgical tape. Also illustrated is the suction cup.

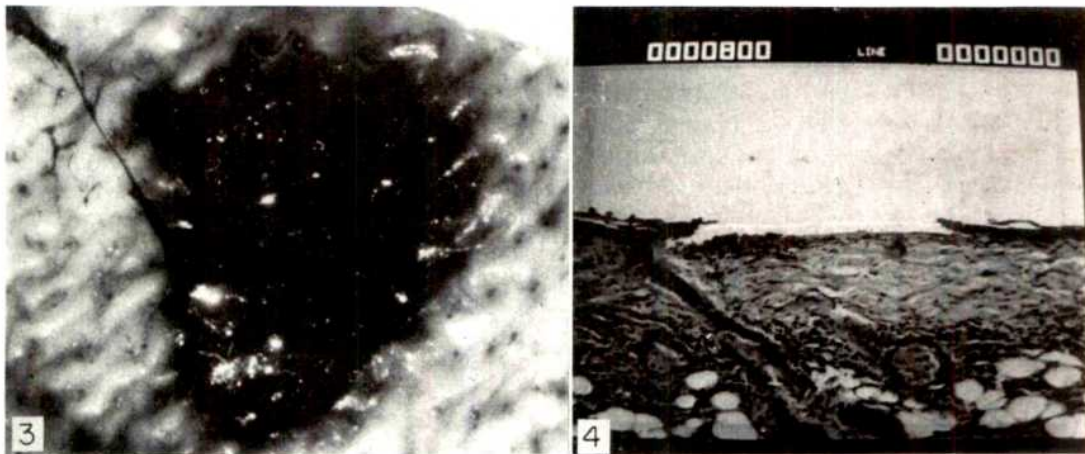


FIGURE 3. The non re-epithelialized area of a 24 h suction blister which is to be measured planimetrically.

FIGURE 4. A cross section of the blistered area as seen on the Quantimet 720. The length of dermis not covered by epidermis is shown by the line which has been drawn by the operator on the screen. The figures displayed in the top left hand corner show the length of the line in picture points.

(see Fig. 3) was then drawn onto graph paper attached to the screen. The true areas of non re-epithelialization were then determined by conversion of the arbitrary units obtained from the graph paper by dividing by a calibration factor (21.2) which had been worked out previously. By measuring the area not covered, immediately after blistering, it is possible to calculate the percentage area re-epithelialized at later times.

(b) *Quantitative microscopy.* At autopsy, 1×1 cm square skin samples which contained the blistered area were removed and placed onto Slek adhesive tape. The samples were placed into formol saline for 24 h, after which time the adhesive tape could be peeled off the rigid flattened skin samples. After routine histological sectioning using a setting of $7 \mu\text{m}$, the sections were stained in haematoxylin and eosin. The blistered sites were sectioned all the way through and every fifth section was examined for the amount of blister base not re-epithelialized. The examination and quantitative evaluation were performed by an Image Analysing Computer (Quantimet 720). Fig. 4 illustrates the measurement and shows the length of dermis not covered by epidermis in a skin section on the Quantimet display screen. The line displayed has been drawn (using the 'image editor' of the Quantimet) by the operator who has decided where uncovered dermis remains. The length of the line is automatically computed in picture points. The figures thus obtained for one-fifth of the sections from one blistered site were automatically summated in picture points which were later converted into absolute length measurements. Every fifth section was evaluated in order to speed the process and because it has been shown in pilot studies that this measurement correlates well with that obtained when all sections are measured. By measuring the area not covered, immediately after blistering, it is possible to calculate the percentage area covered at later times.

Measurement of autoradiographically labelled cells and epidermal thickness

(c) *Labelling index.* Blister sites, which were to be examined for the incorporation of ^3H -thymidine

into DNA, were injected intracutaneously with 10 μ Ci tritiated thymidine (sp. activity = 21 Ci/mmol) in 0.1 ml physiological saline 1 h prior to autopsy. Skin samples were fixed flat, as described in (b), and processed histologically for paraffin serial sectioning at 7 μ m. Autoradiographs of sections containing the cross sectional diameter of the blister were prepared by dipping sections in Ilford K5 nuclear emulsion (exposure time 2–3 weeks). The autoradiographs were then stained in 1% aqueous eosin and differentiated in water such that there was a pale pink epidermis. Measurement of labelled cells per 1 mm length of interfollicular epidermis were performed using a Quantimet 720 (Picton *et al.*, 1976). At least 30 separate 1 mm lengths of interfollicular epidermis were examined per blister site to determine the degree of labelling for each blister examined.

(d) *Epidermal thickness.* Sections of blister sites which were to be examined for epidermal thickness were stained with haematoxylin and eosin such that there was a strong blue epidermis and a pale pink dermis. Epidermal thickness measurements were confined to sections containing the cross sectional diameters of blistered areas. The measurement of epidermal thickness was performed using a Quantimet 720 by measuring the epidermal area per 1 mm length of interfollicular epidermis (Picton *et al.*, 1976). By dividing the area by 1 mm, the epidermal thickness can be calculated. At least 30 separate 1 mm lengths of epidermal area were measured to provide the average epidermal thickness for each blister site.

RESULTS

The results for the measurements of migration are given in Tables 1 and 2 and are illustrated in Figs 5 and 6. It can be seen from both methods that dexamethasone has not significantly affected the rate of migration. As can be seen from Tables 3 and 4 and Figs 7 and 8, dexamethasone significantly reduces the number of autoradiographically labelled cells and epidermal thickening in wound edge and regenerating epithelium.

TABLE 1. Measurement of re-epithelialization of blister sites by direct planimetry in untreated and dexamethasone treated hairless hamsters

Time (h)	% Area of blister base covered	
	Untreated (mean $\pm$ s.e.)	Dexamethasone-treated (mean $\pm$ s.e.)
0	0 (12)	0 (8)
12	6 $\pm$ 11 (12)	
18	22 $\pm$ 11 (12)	
24	16 $\pm$ 10 (20)	26 $\pm$ 8 (11)
30	20 $\pm$ 12 (13)	
36	39 $\pm$ 11 (9)	
42	64 $\pm$ 11 (13)	
48	63 $\pm$ 4 (12)	50 $\pm$ 4 (10)
72		96 $\pm$ 1 (5)

Figures in parentheses denote the number of blisters examined.

TABLE 2. Measurement of re-epithelialization of blistered sites by quantitative microscopy in untreated and dexamethasone treated hairless hamsters

Time (h)	% Blister base re-epithelialized	
	Untreated (mean $\pm$ s.e.)	Dexamethasone-treated (mean $\pm$ s.d.)
0	0 $\pm$ 6 (13)	0 $\pm$ 6 (10)
18	11 $\pm$ 12 (11)	
24	48 $\pm$ 11 (11)	34 $\pm$ 11 (11)
30	92 $\pm$ 4 (12)	
36		85 $\pm$ 1 (2)
42	94 $\pm$ 4 (12)	
48	99 $\pm$ 1 (12)	96 $\pm$ 2 (8)
72		98 $\pm$ 2 (8)

Figures in parentheses denote the number of blisters examined.

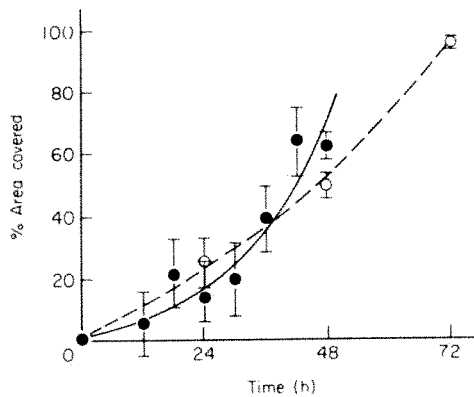


Fig 5

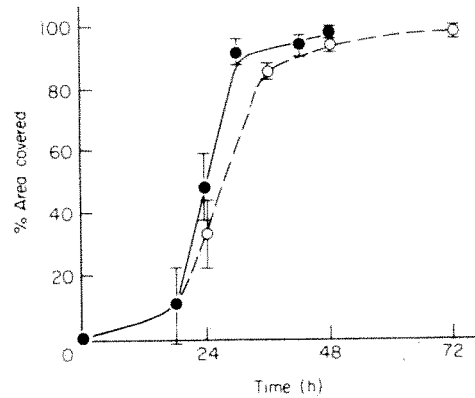


Fig 6

FIGURE 5. The measurement of migration as determined planimetrically. (●) Untreated; (○) dexamethasone-treated.

FIGURE 6. The measurement of migration as determined by quantitative microscopy. (●) Untreated; (○) dexamethasone-treated.

TABLE 3. The effect of dexamethasone on the degree of autoradiographically labelled cells in regenerating epithelium

Time after blistering (days)	No. of labelled cells per 1 mm length of interfollicular epidermis		P
	Untreated (mean $\pm$ s.d.)	Dexamethasone treated (mean $\pm$ s.e.)	
0	5.8 $\pm$ 0.3 (7)	6.6 $\pm$ 0.3 (8)	N.S.
1*	27.0 $\pm$ 2.0 (14)	11.0 $\pm$ 0.4 (9)	< 0.001
2*	19.7 $\pm$ 1.5 (9)	6.3 $\pm$ 0.3 (9)	< 0.001
3	10.6 $\pm$ 1.1 (15)	5.8 $\pm$ 0.5 (14)	< 0.001
5	15.2 $\pm$ 1.4 (12)	12.0 $\pm$ 1.0 (5)	N.S.
7	18.2 $\pm$ 2.1 (9)	10.1 $\pm$ 3.3 (2)	< 0.01
9	27.4 $\pm$ 4.4 (5)	12.1 $\pm$ 2.2 (4)	< 0.001

* These measurements are confined to wound edge epithelium. Figures in parentheses denote the number of blisters examined.

N.S., not significant.

TABLE 4. The effect of dexamethasone on post wounding acanthosis

Time after blistering (days)	Epidermal thickness (μ m)		P
	Untreated (mean $\pm$ s.e.)	Dexamethasone-treated (mean $\pm$ s.e.)	
0	13.1 $\pm$ 0.3 (7)	10.0 $\pm$ 1.0 (8)	< 0.01
1*	31.5 $\pm$ 2.0 (14)	18.0 $\pm$ 0.9 (9)	< 0.001
2*	35.0 $\pm$ 2.6 (9)	19.4 $\pm$ 0.4 (9)	< 0.001
3	23.4 $\pm$ 1.6 (15)	19.3 $\pm$ 0.4 (14)	< 0.001
5	37.3 $\pm$ 2.4 (16)	19.7 $\pm$ 2.6 (5)	< 0.001
7	45.9 $\pm$ 3.0 (9)	17.6 $\pm$ 1.3 (2)	< 0.001
9	44.3 $\pm$ 4.5 (7)	19.5 $\pm$ 0.7 (4)	< 0.001

* These measurements are confined to wound edge epithelium. Figures in parentheses denote the number of blisters examined.

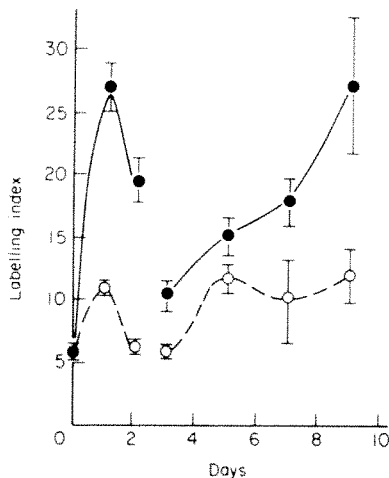


Fig 7

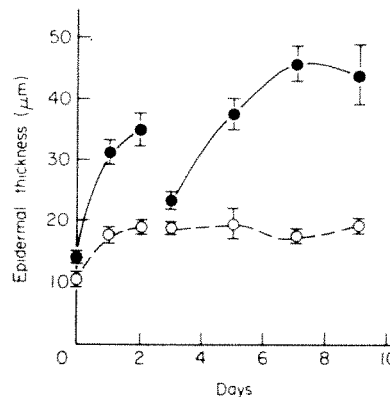


Fig 8

FIGURE 7. Degree of labelling in wound edge and regenerating epithelium. Measurements are confined to wound edge epithelium for days 1 and 2. (●) Untreated; (○) dexamethasone-treated. FIGURE 8. Epidermal thickness in wound edge and regenerating epithelium. Measurements are confined to wound edge epithelium for days 1 and 2. (●) Untreated; (○) dexamethasone-treated.

DISCUSSION

A multiplicity of compounds, dressings and manoeuvres have been claimed to speed or to retard the wound healing process. It seems to us that the only way to determine whether drug administration or other therapeutic activity has an effect on the rate of wound healing is to measure particular aspects of this process in controlled situations. By definition, a wound remains a wound until it is re-epithelialized and although connective tissue repair is of great importance in the regaining of function it is secondary to the provision of a stable and mature epithelium. For this reason we have devoted particular attention to re-epithelialization and its measurement. The model that we describe here evaluates the speed of the development of epithelial cover to an area denuded of epithelium, but we believe that if we can find substances that influence the process we will have a good idea as to their clinical usefulness. Re-epithelialization involves migration of epithelial cells and mitotic activity, with subsequent epidermal thickening, to make good the deficit of epithelium at the wound edges and maturation of the new epithelium.

It is possible from our measurements to produce a general picture of the events taking place in newly regenerating epithelium of an excised suction blister (see Fig. 9). Mitotic activity was observed only in wound edge epithelium over the first 36 h. No activity was observed in the thin sheet of migrating epithelium which emanated from the wound edge and hair follicle epithelium until the denuded area was completely covered. Once covered, mitotic activity in the newly regenerated epithelium steadily increased over the 9 day period examined. Epidermal thickening was observed in wound edge epithelium over the first 2 days and thereafter marked thickening took place over the original wound area.

There seem to have been few other attempts at the measurement of the rate of wound re-epithelialization. Winter (1972) has examined the epidermal regeneration and mitotic activity of wound edge epithelium in the domestic pig by employing a microscope attached to a displacement transducer for recording the lengths of the separate pieces of new epidermis found. Greaves & Skillen (1970) at-

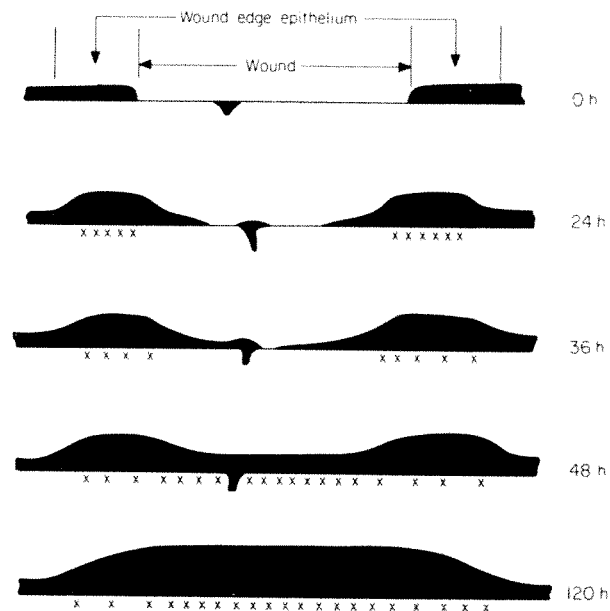


FIGURE 9. Generalized view of the on-going events taking place in regenerating epithelium. The diagram illustrates what is observed when the cross-sectional diameter of the blistered area is examined. Crosses below the epidermis indicate the position of DNA synthetic activity, as observed by labelled cells.

tempted to determine the effect of oral zinc sulphate administration on the rate of re-epithelialization of stasis ulcers by measuring 'two radii. . . one of which lay along the long axis of the ulcer' at different times. Rovee & Miller (1968) employed an ingenious method in which they measured the breaking strength of strips of separated epidermis from the plantar surfaces of guinea-pigs and found that the epidermal breaking strength rapidly increases in the first few days after wounding and that epidermis and keratin are largely responsible for the strength of a wound in the first 5 days. Other methods have employed measurement of the tensile strength of wounds *in vivo* (e.g. Ljungquist & Zederfeldt, 1968) but this and similar methods at best will only measure the development of epithelial cover mixed with connective tissue repair. Other quantitative aspects of epithelialization that have been investigated include mitotic activity at wound edges (e.g. McMinn, 1969); the sequential development of changes in the enzyme histochemical profile (e.g. Rackallio, 1970); and the measurement of epithelial cell migratory activity of guinea-pig epidermis *in vitro* (e.g. Coombes *et al.*, 1974). Clearly these methods at best examine one aspect of epithelialization and we believe that our technique compares favourably with them.

The effects observed in the dexamethasone treated animals in the present study were perhaps not unexpected. The inhibiting effect of glucocorticoids on epidermal mitotic activity is well established (Marks *et al.*, 1971; Marks *et al.*, 1973; Fisher & Maibach, 1971). Winter (1976) has also demonstrated that fluocinolone acetonide, when applied topically to the skin of domestic pigs, retards epidermal regeneration.

Curiously, in practice, there does not seem to be a very great delay in the re-establishment of epithelial cover over wounds of patients on corticosteroids, and indeed the erosions of patients with pemphigus will heal only under the influence of systemic treatment with corticosteroids. However, Sneddon (1968) has noticed that stasis ulcers heal less well when topical corticosteroids are applied.

Clearly many factors are involved in the effect that corticosteroids have on the rate of wound re-epithelialization in individual patients and despite the fact that this paper describes the results obtained using an animal model, we feel that these results indicate that the overall effect of dexamethasone, at least, is likely to be minor when small areas of epithelial loss are involved. The steroid appears not to influence epidermal cell migration, but being anti-mitotic and anti-synthetic, it does delay the full maturation of the newly formed epidermis.

REFERENCES

- COOMBS, V., NISSEN, B.K. & MARKS, R. (1974) The epidermal cell migration promoting activity of serum in guinea-pig skin *in vitro*. *Archiv für Dermatologische Forschung*, **249**, 367.
- FISHER, L.B. & MAIBACH, H.I. (1971) The effect of corticosteroids on human epidermal mitotic activity. *Archives of Dermatology*, **103**, 39.
- GREAVES, M.W. & SKILLEN, A.W. (1970) Effect of long continued ingestion of zinc sulphate in patients with venous leg ulceration. *Lancet*, **ii**, 889.
- LJUNGQUIST, U. & ZEDERFELDT, B. (1968) The effect of high viscous dextran and heparin on wound healing. *Acta Chirurgica Scandinavica*, **134**, 321.
- MARKS, R., HALPRIN, K., FUKUI, K. & GRAFF, D. (1971) Topically applied triamcinolone and macromolecular synthesis by human epidermis. *Journal of Investigative Dermatology*, **56**, 470.
- MARKS, R., PONGSEHIRUN, D. & SAYLAN, T. (1973) A method for the assay of topical corticosteroids. *British Journal of Dermatology*, **88**, 69.
- MCMINN, R.M.H. (1968) *Tissue Repair*. Academic Press, New York.
- PICTON, W., DEVITT, H. & FORGIE, M.A. (1976) Practical applications of the Quantimet 720, an image analysing computer, in the field of investigative dermatology. *British Journal of Dermatology*, **95**, 341.
- RAEKALLIO, T. (1970) *Enzyme Histochemistry of Wound Healing*. Gustav Fischer Verlag, Stuttgart.
- ROVEE, D.T. & MILLER, C.A. (1968) Epidermal role in the breaking strength of wounds. *Archives of Surgery*, **96**, 43.
- SNEDDON, I.B. (1968) Varicose ulcers and use of topical corticosteroids (letter). *British Medical Journal*, **i**, 579.
- WINTER, G.D. (1972) Epidermal regeneration studies in the domestic pig. In: *Epidermal Wound Healing* (Ed. by H.I. Maibach and D.T. Rovee), pp. 71-112. Year Book Medical Publishers, Inc., New York.
- WINTER, G.D. (1976) Epidermal wound healing in corticosteroid treated skin of domestic pig. In: *Mechanisms of Topical Corticosteroid Activity* (Ed. by L.C. Wilson and R. Marks), pp. 61-70. Churchill Livingstone, Edinburgh.

Occupational allergic contact dermatitis due to leatherleaf fern *Arachniodes adiantiformis* (Forst) Tindale

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SUMMARY

Leatherleaf fern has been imported from the United States, Honduras and Puerto Rico to the Federal Republic of Germany since 1966 and cultivated in Germany since 1970/71. It is slowly displacing the floral arrangements with asparagus used until now as filler and background material. This change is due primarily to its better keeping quality. Continuous contact with the leatherleaf fern induced an allergic contact dermatitis of the palms and fingers in a female florist. Patch tests with the leaf revealed the allergic origin of the dermatitis.

Five fractions of a chromatographically separated extract of the fern were tested on sensitized guinea-pigs and on the patient. Only the first fraction gave a positive patch test response. By further separation colourless crystals of the contact allergen could be obtained. Elucidation of the structure is in progress.

The sensitizer could only be detected during sporogenesis of the fern, that means when spore-capsules are present on the surface of the leaves.

Allergic contact dermatitis due to plants is mainly restricted to persons who are professional horticulturists or florists. Less often are farmers, housewives, greengrocers, and amateur gardeners involved. But for those who are affected plant dermatitis is a familiar and often frustrating problem (Schulz & Hausen, 1975; Hausen *et al.*, 1976).

Since 1966 a new decorative and durable ornamental greenery has been introduced to the European flower markets, unknown previously outside Botanical Gardens. This plant has been used as filler and background material in bouquets and table decorations and seems to be well on the way to displacing the floral arrangements with asparagus (*Asparagus setaceus* (Kunth) Jessop and *A. densiflorus* (Kunth) Jessop 'Sprengeri') often used until now.

The plant is the leatherleaf fern *Arachniodes adiantiformis* (Forst) Tindale (Aspidiaceae) (Fig. 1), arhizomous herb native to tropical areas of South America, South Africa and New Zealand; synonyms are *Rumohra adiantiformis* (Forst) Ching, *Polystichum adiantiforme* Forst and *P. capense* J. Smith (Christ, 1910; Foster, 1971; Maatsch, 1972).



FIGURE 1. Frond of leatherleaf fern *Arachniodes adiantiformis* (Forst) Tindale (Aspidiaceae).

FIGURE 2. Surface (backside) of leatherleaf fern leaf during sporogenesis.

Leatherleaf fern is preferred mainly for its better keeping quality and possibly as a change in leaf shape, texture and colour (Conover, 1970). Its leaves are hard as leather; at room temperature it may survive almost 4 weeks without withering*. Leatherleaf fern is produced in Puerto Rico, Honduras and especially in Florida (Volusia County) where it was being cultivated already between 1930 and 1940. Major plantings have been started since 1950. The value of the crop in 1973 has been estimated at more than \$13 million (Conover, 1970). Leatherleaf fern is transported throughout the world by refrigerated trucks, ships and aeroplanes, held at 4–5°C. The export from the United States, Honduras and Puerto Rico to the Federal Republic of Germany in 1975 reached more than 1360 tons, worth DM 8.6 million.†

Other European countries like the Netherlands and Scandinavia receive similar amounts of leatherleaf fern from the United States. The import to Great Britain is somewhat smaller. Hitherto unimportant amounts have been used in France, Italy, Austria and Switzerland.

Attempts at cultivation were started in West Germany in 1970. At a garden exhibition in Cologne (April, 1971) leatherleaf fern was introduced for the first time to cultivators and growers (Maatsch, 1972; Schickedanz, 1974).

Commercial cultivation in the United States is not without problems. It has been necessary to apply insecticides and other control measures against the major insect pest, the leatherleaf fern borer (Short, 1971; Poole, 1973), and against root-lesion nematodes (Hart, 1970; Stokes, 1970). Shortly before shipping, the fern is additionally sprayed with Benlate (= Benomyl) which contains benzimidazol-carbamine-acid-methyl-ester.

* Personal communication of Professor Maatsch, Hannover.

† Based on market reports kindly supplied by Mr W. Helfert, editor of TASPO, Braunschweig.

TABLE 1. Results of patch tests with leatherleaf fern and other plant material (selection). Ten controls also gave ++ to +++ reactions with narcissus stems, but no reactions to leatherleaf fern leaves. 0 = no reaction

Test material	Vehicle	24 h	48 h	72 h
Narcissus petals	Alc.	0	0	0
Narcissus stems	Alc.	+++	+	0
Asparagus stems	Alc.	0	0	0
Cyclamen petals	Aqua dest.	0	0	0
Cyclamen leaf	Aqua dest.	0	0	0
<i>Chrysanthemum indicum</i> L.	Ether extract 1% in acetone	0	0	0
Tulip petals	Isotonic saline	0	0	0
Tulip leaf	Isotonic saline	0	0	0
Fern (native) leaf	Aqua dest.	0	0	0
Leatherleaf fern leaf	Aqua dest.	+	++	+++
Leatherleaf fern F _{1/1}	10% in acetone	0	0	0
Leatherleaf fern F _{1/2}	10% in acetone	+++	++	++

It may be expected that this plant will gain considerable importance in commercial flower sales in the future. Therefore attention should be drawn to allergic phenomena.

CASE REPORT

A woman, aged 21, working in a flower shop since 1973 first developed a dermatitis of her hands in August 1974. Until 1975 she did not consult a physician. During holidays the lesions disappeared. In spring she suffered from a more severe vesicular dermatitis of the palms and fingers. In the shop she had contact regularly with about 60 different flowers and ornamental bushy plants.

Patch tests carried out with compounds of our standard series were negative. Tests with 30 different flowers (petals, stems, leaves, fronds) which the patient handled most frequently as well as with primin, the contact allergen of primula, gave a positive reaction only to narcissus and leatherleaf fern (Table 1). Control tests on 10 unaffected persons were positive to narcissus but negative to the leatherleaf fern.

Because of the recurrence and severity of the dermatitis the patient had to change her job.

Two months later on her birthday she received a bouquet which contained leatherleaf fern as background material. She removed the withered flowers daily, and also cut the fern, on 5 consecutive days. On the 6th day the dermatitis recurred at the same sites.

Animal experiments and chemical investigations

Ten female albino guinea-pigs of the Pirbright white strain were sensitized with a leatherleaf fern extract made by dipping the fronds for 5 min into ether. The residue left after evaporation of the solvent at reduced pressure and low temperature (30°C) was dissolved in acetone to a 10% solution. 0.05 ml of this extract was applied daily to a 2 cm² test area on the clipped and shaved flank of the animals. Five days after the beginning of this procedure a slight erythema developed. On the 17th day, when the reaction had reached a maximum (+++), the treatment was stopped.

Two weeks later challenging was carried out by one epicutaneous application of 0.05 ml of the same solution to the opposite flank. Reading of the test area after 24 and 48 h indicated that all animals had been sensitized to different degrees.

Preliminary chemical investigations of the extract in different solvent systems by analytical thin layer chromatography revealed that the compounds could be separated very well by the system benzene-acetone 100:1.5. With the aid of preparative thin layer chromatography five fractions could be obtained. Patch tests performed with these fractions on the sensitized animals as well as on the patient showed that only the first fraction (F_1) was able to produce an allergic response. By further analytical and preparative separation of F_1 into the fractions $F_{1/1}$ and $F_{1/2}$ it could be demonstrated that only $F_{1/2}$ contained the sensitizer.

A further 40 leatherleaf fern fronds finally yielded 63.2 mg of the pure contact allergen. A further test carried out on the patient and on the sensitized guinea-pigs with this completely purified substance proved that this compound must be regarded as the cause of leatherleaf fern contact dermatitis.

$F_{1/2}$ yielded colourless needles after crystallization from chloroform. Elucidation of the molecular structure is in progress.

Tests with the insecticide Benlate, used for preservation of leatherleaf fern, were negative on the patient as well as on the guinea-pigs; the compound could not be detected chromatographically in the extract.*

During the course of the investigation we noticed that the antigen was not present throughout the whole year in the leatherleaf fern. This assumption was substantiated by studies with thin layer chromatography: the allergenic fraction $F_{1/2}$ could not be detected in extracts from leaves harvested between October 1976 and May 1977, but was isolated in fairly large amounts from plants supplied in June 1977.† These facts indicate that there is a correlation between the occurrence of the allergen and the growth phase of the leatherleaf fern: only when spore-capsules were present on the surface of the leaves (see Fig. 2) could the sensitizer be detected.

During sporogenesis of the fern the $F_{1/2}$ fraction could be found in the leaves, in the sporangia and in the spores themselves, the leaves yielding the greatest amount.

DISCUSSION

This observation shows that introduction of a new unknown exotic plant to the commercial flower market bears a risk of allergic plant dermatitis in those individuals who cannot avoid contact with it. The sensitizing capacity of such plants is often first disclosed 2 or 3 years after their introduction for sale (Hausen & Schulz, 1977).

Reports of allergic dermatitis due to contact with other ferns of the Aspidiaceae family or other fern species could not be found in the medical (*Index medicus*), the chemical or phytochemical (*Chemical Abstracts*) or the biological literature. No indications could be found in the reviews of Wetzel (1938) and Berti & Bottari (1967).

Although the structure of the sensitizing compound is still unknown it may be stated that this substance is a real secondary plant product and not an insecticide or its metabolite.

As leatherleaf fern is becoming continuously more popular further cases of occupational dermatitis in florists may be expected. This fact should be considered in cases of plant sensitization.

Precautions should be taken, especially during the period of sporogenesis because the sensitizer is only present at this time and the spores may cover the fronds, as well as working tables and clothes in the shops, with a fine black dust.

* We are indebted to Schering AG, Berlin, for giving us a sample of Benlate (= Benomyl).

† Dr Barbara Hoshizaki, Professor of Botany at the Los Angeles City College, states that the fronds of the fern are often sterile in spring when the flush of new growth develops (private communication to Professor Maatsch).

ADDENDUM

At time of proof reading a second case of contact allergy due to leatherleaf fern has been recognized in a cultivation plant.

REFERENCES

- BERTI, G. & BOTTARI, F. (1967) Constituents of ferns. In: *Progress in Phytochemistry*, Vol. 1 (Ed. by L. Reinhold and Y. Liwischitz), p. 589. Interscience Publ., London.
- CHRIST, H. (1910) *Die Geographie der Farne*, p. 357. Fischer Verlag, Jena.
- CONOVER, C.A. & LOADHOLTZ, L.L. (1970) Leatherleaf fern production in Florida. *Ornamental Horticulture Report*, 70-1, 1.
- FOSTER, F.G. (1971) *The Gardener's Fernbook*. D.van Nostrand Co., Princeton, New Jersey.
- HART, W.H. & MAGGENTI, A.R. (1970) Granular and soil-spray nematicidal treatment for the control of root-lesion nematode *Pratylenchus penetrans* on leatherleaf fern. *Plant Disease Reporter*, 54, 1016.
- HAUSEN, B.M., KETELS-HARKEN, H. & SCHULZ, K.H. (1976) Berufsbedingte Inhalationsallergie durch Pollen von *Euphorbia fulgens* KARW. *Deutsche Medizinische Wochenschrift*, 101, 567.
- HAUSEN, B.M. & SCHULZ, K.H. (1977) Contact dermatitis due to 'Croton' (*Codiaeum variegatum* (L.) A.Juss. var. *pictum* (Lodd.) Muell. Arg.). *Contact Dermatitis*, in press.
- MAATSCH, R. (1972) Der Lederfarn als Schnittgrünlieferant. *Die Gartenwelt*, 72, 49.
- POOLE, R.T. & CONOVER, C.A. (1973) Influence of dolomite and micronutrients on yield of leatherleaf fern. *Proceedings of the Florida State Horticultural Society*, 86, 372.
- POOLE, R.T. & CONOVER, C.A. (1973) Influence of shade, nitrogen, and potassium levels on production and elemental composition of leatherleaf fern. *Proceedings of the Tropical American Society for Horticultural Science*, 17, 385.
- SCHULZ, K.H. & HAUSEN, B.M. (1975) Kontaktekzeme durch Pflanzen und Hölzer. *Hautarzt*, 26, 92.
- SHORT, D.E., DRIGGERS, D.P., KUITERT, L.C. & ROBERTS, C.R. (1971) Control of leatherleaf fern borer. *The Florida Entomologist*, 54, 325.
- SCHICKEDANZ, F. (1974) Leaf blight of imported leather fern caused by fungus of the genus *Cylindrocladium* Morgan. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 81, 528.
- STOKES, D.E. & LAUGHIN, C.W. (1970) Control of *Pratylenchus penetrans* on leatherleaf fern transplants. *Plant disease Reporter*, 54, 287.
- WETZEL, K. (1938) Chemie und Stoffwechsel. In: *Manual of Pteridology* (Ed. by F. Verdoorn), p. 347. M. Nijhoff, The Hague.

Histochemical demonstration of histamine in primary cutaneous inflammation of the rat

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SUMMARY

A primary (non-allergic) inflammatory response was elicited in the skin of albino rats by exaggerated application of sodium laurate solution, and the liberation of histamine, a known mediator of cutaneous inflammation in many species, was studied by a histofluorimetric stain specific for this amine which forms a complex with orthophthalaldehyde. Within the first 2 h after irritation both the epidermis and the upper dermis exhibited widespread diffuse, extracellular yellow fluorescence of the histamine-orthophthalaldehyde complex; dilated blood vessels and many fibroblasts were also fluorescent. The extracellular and vascular staining increased in intensity up to about 6 h and then gradually faded. By about 12 h after irritation, yellow histamine fluorescence was located only as a narrow band in the upper dermis close to the dermo-epidermal junction. The fluorescence was probably contained within cells, tentatively identified as leukocytes. Other than occasional mast cells in deeper regions of the dermis, no other histamine-fluorescent cells were seen throughout the response.

None of these features was observed in normal, control rat skin treated only with distilled water, and routinely we failed to observe more than just an occasional fluorescent mast cell in irritated or control skin alike. The morphological features of the inflammation closely resembled those described by others for chemical irritation of skin. This study indicates the presence of histamine in the early stages of cutaneous inflammation of the rat, and suggests routes of its dispersal at later stages, when other inflammatory mediators are known to be involved.

Although histamine is known to be an early mediator of the inflammatory response in many species (Spector & Willoughby, 1968), in the rat it is reported to possess only slight potency as a vascular permeability factor compared with other known mediators such as 5-hydroxytryptamine or bradykinin (Wilhelm, 1973). In 1966 Juhlin & Shelley described a specific histofluorimetric stain for histamine which depended upon the formation of a fluorescent complex between endogenous histamine and orthophthalaldehyde. These authors demonstrated that rat mast cells *in vitro* and other histamine-rich tissues were fluorescent by this technique. Later, Juhlin (1967) used the technique to demonstrate the increased amounts of histamine in the skin of patients suffering from various inflammatory skin dis-

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eases. Also in 1967 Ehinger & Thunberg, using orthophthalaldehyde histofluorescence by a slightly different technique, showed that in rat mesentery, mast cells which were positively identified by toluidine blue metachromasia were very fluorescent.

It is known that exaggerated application of sodium laurate solutions to rat skin may elicit a primary inflammatory response (Prottey & Hartop, 1973; Prottey & Ferguson, 1975). In order to establish whether histamine is released in the mediation of primary inflammation, the skin of rats, similarly irritated, was examined by the histofluorimetric stain for histamine.

MATERIALS AND METHODS

Animals

Rats were all weanlings, 4–6 weeks of age and weighing about 80 g, of the Colworth/Wistar albino strain, maintained on Spital diet and water *ad libitum*.

Treatment with sodium laurate

Sodium laurate was prepared by adding an equivalent amount of NaOH to pure lauric acid (Koch-Light Laboratories Ltd., Colnbrook, Bucks) dissolved in ethanol/water (1:1 v/v). Solvent was then removed by flash evaporation and lyophilization. Solutions (0.25 M) were made up in distilled water and the pH was found to be 9.4–9.8. The back and flanks of animals were shaved with electric clippers on the day prior to application of 0.2 ml of a 0.25 M solution of sodium laurate which was then gently massaged with a smooth glass rod for several seconds. At 7.5, 15 and 22.5 min the treated surfaces were moistened by addition of further amounts of the solution, with massaging. After 30 min from the start of treatment, when a total amount of 0.4 ml had been applied, the animals were rinsed with warm distilled water from a wash bottle and gently patted dry with paper tissues. They were then killed at precise times measured from the end of the treatment period. Control animals were treated with 0.4 ml distilled water.

Assessment of permeability changes

Rats received 0.3 ml of 1% Evans Blue (B.D.H., Poole, Dorset) in sterile 0.9% saline by intracardial injection under light anaesthesia. The degree of skin blueing, an indication of increased vascular permeability to plasma proteins, was assessed visually on the external surface exactly 10 min later.

Histofluorimetric staining for histamine

The method of Juhlin (1967) was followed. Freshly excised fragments of skin (measuring 7 mm in diameter) were immediately incubated in Ringer's-glucose solution, containing 1% orthophthalaldehyde (Sigma, London) for 2 h at 37°C. After incubation, the skin fragments were frozen in hexane cooled to -70°C and sections cut with a cryostat microtome. The 8 µm sections were placed on a glass slide and mounted in alkaline gelatin medium, consisting of 70 ml 1% NaOH, 15 g gelatin and 15 ml glycerol. With samples of rat pleura and mesentery a preliminary incubation for 3 min in Tyrode's solution, pH 11, at 50°C was necessary (Juhlin & Shelley, 1966) before the incubation with orthophthalaldehyde, otherwise the mast cells of these tissues would not show the yellow fluorescence. The stained histamine was detected using a Leitz Labolux microscope fitted with fluorescent optics (2 mm UGI exciting filter and a K430 UV suppression filter). It was not possible to stain further these sections for histological examination as, after incubation with orthophthalaldehyde, skin samples turned black (Ohman & Shelley, 1969) and this blocked the uptake or development of subsequent stains. Thus, routine staining for assessment of morphological change in irritated skin was done on separate skin samples from adjacent regions.

Juhlin & Shelley (1966) and Ehinger & Thunberg (1967) reported that the yellow fluorescence of the

orthophthalaldehyde complex was specific for histamine. This was confirmed by spotting solutions of many other compounds (100 µg in 0.05 ml saline) on to chromatography paper, exposing to orthophthalaldehyde, and then irradiating with ultraviolet light. Only histamine and histidine gave an intense yellow stain. Glutathione gave a blue fluorescence, agmatine and arginine gave a duck-egg green fluorescence, spermidine and metabolites of histamine such as methyl imidazole and imidazole acetic acid did not fluoresce.

Histological examination

Samples of whole skin were fixed in formol saline and stained with either haematoxylin and eosin, toluidine blue, Giemsa, azo-eosin or carbol chromatrope stains.

RESULTS

Morphological changes following sodium laurate irritation

Visible and vascular changes in sodium laurate-treated rat skin resembled those described by Steele & Wilhelm (1966) for inflammation in guinea-pig skin produced by chemicals. Increased vascular permeability was monophasic and of the 'delayed' type, as described by these authors, namely, maximal at 6–8 h but barely discernible by 24 h. The development and abatement of a moderate erythema also approximately corresponded to these times. Not all rats reacted uniformly to the irritant. Some showed maximum skin blueing as early as 2 h after application of sodium laurate (usually those sustaining clipper damage during shaving by abrasion of the stratum corneum) whereas others showed no increase in vascular permeability for up to 24 h. Histologically, the changes in irritated skin were similar to those described by Steele & Wilhelm (1970) for solvent-treated guinea-pig skin and by Medenica & Rostenberg (1971) for DNCB-irritated guinea-pig skin. Thus, only brief descriptions are given below as background to the histofluorimetric staining of histamine.

Histofluorimetric staining of histamine during the inflammatory response

Control (water-treated) rat skin (Fig. 1). The stratum corneum showed a yellowish-brown colour and the whole of the epidermis gave a uniform bluish-white fluorescence, as did hair keratin. In the dermis, fibroblasts were faintly blue. There was no yellow histamine fluorescence in either the epidermis or the dermis. Only occasional yellow fluorescent mast cells were seen in the upper part of the dermis, and in the lower regions they were usually in groups of 2 or 3 localized near arterioles. Cutaneous blood vessels were not yellow fluorescent, unlike the vascular bed of rat tongue (Juhlin & Shelley, 1966).

0–2 h. Few morphological changes were seen in the epidermis, namely, slight perinuclear vacuolation, nuclear pyknosis and increased eosinophilic staining of the cytoplasm. There was loss of some superficial stratum corneum, presumably due to the physical washing action with sodium laurate. There were no observable changes in the dermis.

The histofluorimetric stain for histamine showed differences compared with controls during this time (Fig. 2). The whole of the epidermis exhibited a very faint diffuse yellow fluorescence that masked the blue fluorescence of control skin. The upper dermis also showed a faint, extracellular, diffuse yellow fluorescence but in particular, many yellow-fluorescing cells were seen. It is thought that these were fibroblasts which, presumably, had scavenged liberated histamine. It was impossible to determine whether any of these fluorescent cells were mast cells; separate staining with toluidine blue indicated that few mast cells were present in the upper dermis, and these were small in size compared with those associated with blood vessels in the lower dermis. In some samples examined at 2 h occasional dilated and fluorescent blood vessels were seen.

2-6 h. Histologically the epidermis showed much loss of cellular detail and stained strongly with eosin. When discernible, cell nuclei of the stratum basale were shrunken and the dermo-epidermal junction was ill-defined or absent. Dermal blood vessels were dilated with leukocytes emigrating from them. The numbers of neutrophils and lymphocytes greatly increased, but eosinophils only moderately so. Mast cells were sparse, some close to the larger blood vessels of the lower dermis showing signs of degranulation. Leukocytic emigration was confined to regions of the dermis underlying the most degenerated epidermis.

The diffuse yellow fluorescence of histamine by 6 h could be seen throughout the whole of the epidermis and the upper 200 μm or so of the dermis, and on average was at its maximum intensity at this time. Fluorescent cells throughout the dermis were seen, but separate staining with toluidine blue failed to identify more than just an occasional mast cell amongst those in the upper dermis. Dilated and strongly fluorescent blood vessels were observed throughout the dermis.

6-10 h. Generally by about 8 h the diffuse yellow fluorescence of the epidermis and upper dermis was less intense, although blood vessels, including capillaries, were still dilated and fluorescent. By about 10 h the diffuse fluorescence was confined to the upper dermis but fibroblasts no longer exhibited their hitherto bright fluorescence. Toluidine blue staining confirmed the presence of a few mast cells scattered throughout the dermis, but the responses of individual animals were so varied that it was not possible to say whether the number of mast cells had altered relative to normal skin. Blood vessels were still dilated and fluorescent.

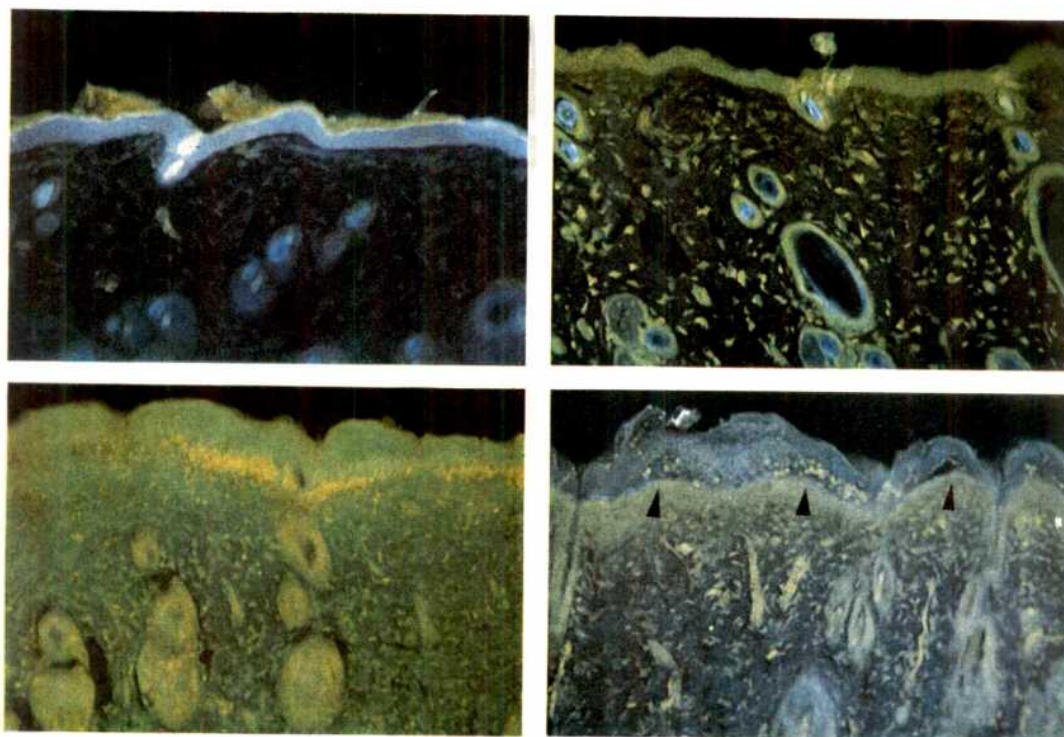
10-16 h. The irritated epidermis lost much structural detail, and was heavily infiltrated with a leukocytic exudate, within which mainly neutrophils could be identified, but also eosinophils, basophils and lymphocytes were seen. As many of these cells had degenerated, and nuclear detail was lacking, precise identification was not possible. In the upper dermis there were many leukocytes, collagen was swollen with separated fibrils and venules were still dilated, but no longer engorged with leukocytes. During this period the histofluorimetric stain faded completely from the fibroblasts and dilated blood vessels, and fluorescent mast cells could not be seen. However, a band of bright yellow fluorescence was observed within the leukocytic exudate, immediately beneath the dermo-epidermal junction (Fig. 3). Giemsa and carbol chromotrope staining of sections consecutive to those incubated with orthophthalaldehyde showed neutrophils and eosinophils in this region, but it could not be ascertained whether the fluorescence was contained within a specific cell type. From about 12 h onwards new epidermis was seen to grow out from the follicular epithelium and underneath the fluorescent band.

16-24 h. Histologically the epidermis was replaced by a thick crust of exudate containing many degenerated leukocytes. Histofluorimetric staining showed that the narrow band of fluorescence which seemed, but could not be proved, to be within leukocytes, had moved higher into the exudate. As regrowth of epidermis became complete, effectively separating the exudate from the underlying dermis, all cell detail in the exudate was lost. Fig. 4 illustrates the fluorescence at 24 h.

24-72 h. The fluorescent band persisted in the exudate, but generally by about 3 days this had been sloughed off, and the skin was replaced by new, rather acanthotic epidermis that differed little from normal skin (Fig. 1) with regard to staining with orthophthalaldehyde, namely, only occasional mast cells in the vicinity of deeper lying vessels were fluorescent.

Specificity of the yellow fluorescence for histamine in vivo

Solutions of histamine, glutathione, 5-hydroxytryptamine and histidine (100 μg in 0.05 ml saline) and compound 48/80 (1 μg in 0.05 ml saline) were injected intradermally into rats and skin sections stained



Histofluorimetric staining for histamine in rat skin.

FIGURE 1. Untreated control skin. The whole of the epidermis shows a blue fluorescence and the stratum corneum, when present, a yellowish-brown colouration ($\times 50$).

FIGURE 2. Two hours after end of treatment with sodium laurate. Diffuse yellow fluorescence in epidermis masks the blue fluorescence of Fig. 1. Many fibroblasts throughout dermis fluoresce yellow ($\times 50$).

FIGURE 3. After 16 h. Epidermis replaced by exudate. Most of the diffuse fluorescence has disappeared and is seen in a narrow band of leukocytes beneath what was once the dermo-epidermal junction ($\times 50$).

FIGURE 4. After 24 h. New epidermis (shown by black arrows) that has grown out from hair follicles. Band of yellow fluorescence, presumably within leukocytes, in the epidermal exudate, which is subsequently lost by sloughing ($\times 50$).

with orthophthalaldehyde exactly 10 min later, when maximum vascular permeability was observed with Evans Blue dye. Only histamine gave extensive yellow fluorescence in the panniculus carnosus and panniculus adiposus, presumably the site of the injected solution.

Several large blood vessels associated with the muscle were brightly fluorescent, but the overlying collagen, the upper dermis and the epidermis were negative. A weaker fluorescence was seen with 5-hydroxytryptamine presumably due to release of endogenous histamine, as the former amine does not fluoresce yellow with orthophthalaldehyde (see Methods).

Injection into rats of compound 48/80, a histamine liberator, produced swelling and reddening of the skin, and staining with OPT showed a diffuse yellow fluorescence throughout the sections. However, the skin of rats which were injected with sodium cromoglycate, which blocks the release of histamine, before the injection of compound 48/80 did not show the diffuse yellow fluorescence.

In some of the controls of injected saline, a narrow shaft of weak yellow fluorescence was seen extending downwards through the dermis. It is thought that these sections showed the needle injection site; presumably, the physical trauma of injection caused local release of endogenous histamine.

DISCUSSION

We have examined the occurrence of histamine in inflammation of rat skin by a histofluorimetric stain first described by Juhlin & Shelley (1966). These authors demonstrated histamine in mast cells, platelets, gastric mucosa and the vascular bed of the tongue of laboratory rodents. Subsequently, orthophthalaldehyde fluorescence has been used to demonstrate histamine in various normal and diseased tissues of man (Juhlin, 1967), rat mesenteric mast cells (Ehinger & Thunberg, 1967) and the stomach and pancreas of the rat (Brody *et al.*, 1972). Although here we give no categorical proof that the yellow fluorescence observed is due to histamine solely, data of Ehinger & Thunberg (1967) as well as our own on the specificity of the stain for histamine allow us to conclude tentatively that release and translocation of histamine is a feature of primary cutaneous inflammation of the rat.

We found routinely that the originally published method for producing the histamine-orthophthalaldehyde complex *in situ* (Juhlin & Shelley, 1966) was quite satisfactory and reproducible. This involved incubation of tissue fragments in 1% solutions of orthophthalaldehyde for 2 h.

Brody *et al.* (1972) introduced a modification of brief exposure of frozen-dried tissue sections to humid orthophthalaldehyde vapour, to obviate diffusion artefacts that were suggested to be a problem in the original incubation method. In our hands, however, the method of Brody *et al.* (1972) was quite unreproducible. Juhlin & Shelley (1966) also described the use of 1% orthophthalaldehyde in xylene at -25°C with fresh frozen sections of tissue. With this technique we obtained similar results to those using the incubation procedure, but the resultant histamine fluorescence was always less stable, and routine photography of the sections was not possible.

With toluidine blue staining, we observed very few mast cells in the skin of normal rats, mainly in lower regions of the dermis in small groups associated with the larger blood vessels, but with occasional ones in the upper dermis. The presence of many mast cells in rat ear skin has been reported (Riley, 1959) but their density in other areas of rat skin is much less (Parish, 1964). With the orthophthalaldehyde stain we were unable to detect fluorescence in these cells in normal rat skin routinely (see Fig. 1). Also, with rat mesentery and pleura we were unable to show the yellow fluorescence of the unusually large numbers of mast cells of these tissues, unless a pre-incubation in alkaline Tyrode's solution was performed prior to that with orthophthalaldehyde, as suggested by Juhlin & Shelley (1966). This preincubation was not successful for skin, however, and suggests that either rat cutaneous mast cells *in situ* are very sparse, or are not readily revealed by this technique, unlike their peritoneal counterparts *in vitro* or blood basophils (Juhlin & Shelley, 1966). However, we found that the diffuse, extracellular histamine was readily demonstrable, as was that either in cutaneous vessels or engulfed by cells.

We never found firm evidence of the diffusion artefacts discussed by Brody *et al.* (1972). Diffuse histamine fluorescence was only seen at specific (early) times of the inflammatory reaction. In confirmation, it was seen that with smears of normal rat peritoneal mast cells, or sections of rat tongue (which exhibits a highly fluorescent vascular bed) there was no diffuse surrounding fluorescence. The only other instances of diffuse histamine staining in skin samples we observed were when either cut edges of tissues or needle injection sites were examined—in these instances it was assumed that the physical traumata of excision or injection had caused histamine to be released.

The finding of histamine fluorescence in the epidermis as well as the dermis after 2 h (Fig. 2) was unexpected. There is no firm evidence that histamine is an important mediator of the epidermal response to primary irritation, although Steele & Wilhelm (1970) suggested that mediators (e.g. histamine) may be released from damaged epidermal cells followed by rapid vasodilation of the dermal vessels within the first 2 h of irritation in guinea-pigs. This may have been derived by diffusion from the dermis, however. The epidermal histamine fluorescence persisted for up to 8 h, being maximal at 4–6 h.

The diffuse, extracellular histamine released into the dermis after irritation appeared to be removed subsequently by two distinct routes.

As early as 2 h after irritation many fibroblasts were fluorescent (Fig. 2), by 10–14 h their fluorescence was weak and by 24 h it had disappeared. It is possible that fibroblasts rapidly took up some of the histamine liberated in the dermis as a consequence of inflammation and metabolized it to non-fluorescent (and non-vasoactive) products—indeed, these cells may be important in the subsequent removal and inactivation of pharmacological mediators. Parish (1964) showed that granules released from rat and guinea-pig mesentery mast cells following anaphylaxis rapidly adhered to and aggregated on the plasma membrane of fibroblasts, and also within the cytoplasm where granule breakdown was observed. This 'scavenging' role of fibroblasts for histamine is quite novel and studies with fibroblasts maintained in cell culture with radioactive histamine are in hand to investigate this phenomenon further.

After about 12 h following application of the irritant the diffuse yellow fluorescence in the dermis was concentrated immediately below the dermo-epidermal junction, and by about 16 h was found in a narrow band within an exudate amongst which neutrophils, eosinophils, lymphocytes, red blood cells and fibroblasts were tentatively identified (precise identification was difficult, despite the use of specific stains, as most of these cells had degenerated). From its speckled appearance under the microscope, it would seem that this band of fluorescence was actually localized within cells comprising the exudate, but this could not be confirmed, as post staining of tissue already exposed to orthophthalaldehyde was unsuccessful.

Whereas the fluorescence that was taken up by the fibroblasts at early stages of the inflammation rapidly subsided, that fluorescence localized as a band in the exudate persisted, suggesting that the histamine was not degraded. This is not unreasonable as most cells of the exudate had degenerated, and would have lost metabolic activity. At later stages, when new epidermis had grown under the original site of inflammation, the exudate (still containing fluorescence) was sloughed off.

Several roles have been attributed to histamine in inflammation, for example; the role of this amine as a vascular permeability factor is well documented (Spector & Willoughby, 1968; Wilhelm, 1973); in the attraction of eosinophils into tissues (Archer, 1963); in the trapping of eosinophils and neutrophils in tissues (Parish, 1970); in wound healing (Sheldon & Buer, 1960); and, in concert with cyclic nucleotides, in the regulation of inflammatory and immune responses of various types of leukocytes (Bourne *et al.*, 1974). The present study is a demonstration of the localization and fate of histamine released in an experimental cutaneous inflammation of the rat *in vivo*, namely, its presence extracellularly and bound to blood vessels, within fibroblasts at intermediate times, and probably within leukocytes in an epidermal exudate that is eventually sloughed off.

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REFERENCES

- ARCHER, R.K. (1959) Eosinophil leucocytes and their reactions to histamine and 5-hydroxytryptamine. *Journal of Pathology and Bacteriology*, **78**, 95.
- BOURNE, H.R., LICHTENSTEIN, L.M., MELMON, K.L., HENNEY, C.S., WEINSTEIN, Y. & SHEARER, G.M. (1974) Modulation of inflammation and immunity by cyclic AMP. *Science*, **184**, 19.
- BRODY, M.J., HAKANSON, R., OWMAN, C. & SUNDLER, F. (1972) An improved method for the histochemical demonstration of histamine and other compounds producing fluorophores with o-phthalaldehyde. *Journal of Histochemistry and Cytochemistry*, **20**, 945.
- EHINGER, B. & THUNBERG, R. (1967) Induction of fluorescence in histamine-containing cells. *Experimental Cell Research*, **47**, 116.
- JUHLIN, L. (1967) Localization and content of histamine in normal and diseased skin. *Acta dermato-venereologica*, **47**, 383.
- JUHLIN, L. & SHELLEY, W.B. (1966) Detection of histamine by a new fluorescent o-phthalaldehyde stain. *Journal of Histochemistry and Cytochemistry*, **14**, 525.
- MEDENICA, M. & ROSTENBERG, A. (1971) A comparative light and electron microscopic study of primary irritant contact dermatitis and allergic contact dermatitis. *Journal of Investigative Dermatology*, **56**, 259.
- OHMAN, S. & SHELLEY, W.B. (1969) Orthophthalaldehyde staining of coiled and uncoiled intraepidermal sweat ducts. *Journal of Investigative Dermatology*, **53**, 29.
- PARISH, W.E. (1964) The distribution of mast cells and the possible function of some of their pharmacological agents. In: *Biological Aspects of Occlusive Vascular Disease* (Ed. by D.G.Chalmers and G.A.Gresham), p.84. Cambridge University Press.
- PARISH, W.E. (1970) Investigations on eosinophilia. *British Journal of Dermatology*, **82**, 42.
- PROTTEY, C. & HARTOP, P.J. (1973) Changes in glycerolipid metabolism in rat epidermis following exaggerated washing with soap solutions. *Journal of Investigative Dermatology*, **61**, 168.
- PROTTEY, C. & FERGUSON, T.F.M. (1975) Factors which determine the skin irritation potential of soaps and detergents. *Journal of the Society of Cosmetic Chemists*, **26**, 29.
- RILEY, J.F. (1959) *The Mast Cells*. Livingstone, London.
- SHELDON, W.H. & BUVER, H. (1960) Tissue mast cells and acute inflammation in experimental cutaneous mucormycosis of normal, 48/80-treated and diabetic rats. *Journal of Experimental Medicine*, **112**, 1069.
- SPECTOR, W.G. & WILLOUGHBY, D.A. (1968) *The Pharmacology of Inflammation*, p.22. The English Universities Press, London.
- STEELE, R.H. & WILHELM, D.L. (1966) The inflammatory reaction in chemical injury. I. Increased vascular permeability and erythema induced by various chemicals. *British Journal of Experimental Pathology*, **47**, 612.
- STEELE, R.H. & WILHELM, D.L. (1970) The inflammatory reaction in chemical injury. III. Leucocytosis and the histological changes induced by superficial injury. *British Journal of Experimental Pathology*, **51**, 265.
- WILHELM, D.L. (1973) Chemical mediators. In: *The Inflammatory Process* (Ed. by B.W.Zweifach, L.Grant and R.T.McCluskey), 2nd edn, vol. II, p.251. Academic Press, New York and London.

Antiserum to the cell surface of oral epithelial cells: relationship to pemphigus antiserum

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SUMMARY

Antisera to human oral epithelial cells were produced in rabbits and found to be specific for epithelial type cells of man, guinea-pig and rabbit. Binding of the antisera to human oral epithelial cells was not affected by pre-incubation in concanavalin A, trypsin or neuraminidase, nor by pre-incubation of the antisera with fetuin. The antisera would appear to differ from pemphigus antiserum.

In pemphigus, antibody can be detected to both the surface of the stratified squamous epithelial cell and material in the intercellular area in man and in several laboratory animals (Chlorzelski *et al.*, 1968; Beutner *et al.*, 1970). Although the nature of the antigen or antigens is unknown, inhibition of antibody binding can be achieved with concanavalin A (con.A) (Van Lis & Kalsbeek, 1975), suggesting that the pemphigus antigen enclosed the con.A binding sites. Pemphigus-like antibodies have been produced in rabbits by immunization with crude epithelial extracts (Grob & Inderbitzin, 1967a; Ablin, 1969). In this study human oral epithelial cells, derived without enzymatic treatment, were used in an attempt to produce pemphigus antibodies. The antisera were assessed with human oral epithelial cells released with and without the use of enzyme, and cells from 2 laboratory animals were also examined. The characteristics of the antisera were compared with the known characteristics of pemphigus antisera.

MATERIALS AND METHODS

Derivation of cells

Oral epithelial cells were obtained by trypsinization (Dolby, 1969) or autolytic dispersion (Milne & Dolby, 1972). Samples of the cells were examined by electron microscopy. Skin, oral mucosal and small intestinal cells were derived with 0.25% trypsin, and striated and cardiac muscle cells with 0.05% collagenase (Sigma Ltd.) from rabbit and guinea-pig tissue. Cover-slip cultures of human fetal cells were examined *in situ*.

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Production of antisera (EC antisera)

White New Zealand rabbits (3) were injected twice, with 3×10^6 autolytically released oral epithelial cells in Freund's complete adjuvant (FCA—Difco), bled at 30 days and the sera decomplexed. Normal rabbit sera (NRS) were obtained from 6 rabbits (> 8 weeks old) of the same breed and control sera (FCA only) from 2 rabbits.

Absorption of antisera

Aliquots of EC antisera, NRS and control sera were absorbed twice with double the volume of packed pooled red cells, A + ve and B + ve. The EC antisera were also absorbed, in additional experiments with packed blood cells from the donor of the epithelial cells. These EC antisera (donor absorbed) were also incubated for 1 h at 20°C with fetuin (Sigma Ltd.) at 4 mg/ml to 0.5/ml of EC antisera.

Immunofluorescence

Cells were examined by immunofluorescence (Dolby, 1972) using EC antisera and fluorescent goat anti-rabbit globulin (Wellcome Reagents Ltd.) centrifuged at 20,000 g prior to use.

Enzyme, lectin and detergent treatment of oral epithelial cells

Trypsinized oral epithelial cells were incubated (37°C for 30 min) with, all in PBS, (a) con.A (Sigma Ltd.), 10, 5, 1, 0.5, 0.1 mg/ml (b) neuraminidase (Koch Light Laboratories), 500, 50, 5, 1, 0.1 units/ml (c) protease Type VI (Sigma Ltd.) at 5, 1, 0.5, 0.1, 0.05, 0.01 mg/ml (d) Tween 80 (Koch Light Laboratories) 1/10, 1/100, 1/1000, 1/10,000, 1/100,000.

RESULTS

The cells examined by electron microscopy had the features of oral epithelial cells. The EC antisera absorbed with allogeneic packed red blood cells produced surface fluorescence with oral epithelial cells (0.1%, 0.25% trypsin and autolysis) to a titre of 1/64–1/128. The NRS and control sera produced a similar pattern of fluorescence (both 1/4) and the PBS was negative. The packed red blood cells from the second absorption were negative.

The EC antisera absorbed with donor packed blood cells produced surface fluorescence with oral epithelial cells (0.25% trypsin) to a titre of 1/256. The white blood cells of the packed blood cells fluoresced after the first absorption only. With both the EC antisera (allogeneic and packed blood cell absorbed) and NRS, squamous cells had a fine continuous rim of fluorescence whereas basal or prickly cells had a brighter, irregular edge fluorescence.

The fluorescence did not appear to be affected by preincubation of the oral epithelial cells in con. A or neuraminidase. With protease (5, 1, 0.5 mg/ml) the cell wall was apparently disrupted and fluorescence was lost from these areas. At 0.1 and 0.05 mg/ml, fluorescence was uniformly reduced but again the cell wall was apparently damaged. Tween 80 at 1/10–1/1000 completely ablated the surface fluorescence. Incubation of the antiserum with fetuin resulted in the formation of a precipitate and the supernatant (20,000 g) bound to the same titre as before. In the fetal cell cultures only the epithelial cells showed fluorescence (1/64). The animal cell suspensions from skin, oral mucosa and small intestine contained epithelial cells which were positive (Table 1) whereas the striated and cardiac muscle samples were negative.

DISCUSSION

The EC antisera appeared to possess specificity for the surface of epithelial type cells found in man

TABLE 1. The titre of positive fluorescence observed with the antiserum using human, rabbit and guinea-pig tissues derived by trypsin (t) or collagenase (c) treatment or without enzyme. Absorption with A and B+ human blood cells (AB) or donor packed blood cells (DPBC)

Tissue examined	Titre of positive fluorescence		
	Human	Rabbit	Guinea-pig
Gingiva, gingiva (t)	1/64, 1/256 (AB) (DPBC)		
Amnion cell suspension			
(a) Epithelial cells	1/64 (AB)		
(b) Fibroblasts	—		
Tongue (t)		256 (DPBC)	256 (DPBC)
Buccal mucosa (t)		64 (DPBC)	256 (DPBC)
Small intestine (t)		256 (DPBC)	256 (DPBC)
Skin (t)		256 (DPBC)	256 (DPBC)
Cardiac muscle (c)		— (DPBC)	— (DPBC)
Striated muscle (c)		— (DPBC)	— (DPBC)

and the two laboratory animals examined and apparently could be made free of antibody to antigens shared by blood cells and epithelial type cells. Trypsin treatment of the cells did not impair the fluorescence observed with the EC antisera. In these respects the EC antisera were similar to pemphigus antisera, reacting with antigens of the surface of epithelial cells from several sources (Beutner, 1970) which are not of blood group type (Grob & Inderbitzin, 1967b; Ablin, 1969) and are not affected by weak (0.3%) trypsinisation (Bridgen & Amos, 1975). It is not certain whether the antisera represent an enhancement of the naturally occurring rabbit antibodies to epithelial cells or a new response to additional antigenic determinants. However, normal animal sera have been shown to possess antibody to cell surface carbohydrate (Sela *et al.*, 1975) which is significantly inhibited by prior absorption of the sera with fetuin. No significant inhibition with fetuin was observed with the antisera produced in this investigation. Although inhibition of binding could be achieved by pre-incubation of the cells in protease or Tween 80, no inhibition was observed after pre-incubation of the cells in con.A. The appearance of the cells after treatment with protease and Tween 80 would suggest that integral membrane proteins had been removed from the cell wall (Singer & Nicholson, 1972). Failure to inhibit binding with con.A would suggest that this antigen was not prominent amongst the immunizing antigens and that it is possibly not generally accessible for antigenic stimulation by the intact cell. Conversely, since pemphigus antigen can be demonstrated on cells released by trypsinization with pemphigus antiserum (Bridgen & Amos, 1975) it is possible that additional antigen sites on the intact cell normally evoke higher titre antibodies and impair binding to pemphigus antigen sites.

During the preparation of this manuscript a report of the production of rabbit antisera to guinea-pig epithelial cells was published (Takigawa & Imamura, 1977) in which differences were observed between the rabbit antisera and pemphigus antisera with respect to complement mediated cytotoxicity and trypsin sensitivity. Our investigation would tend to support the findings of Takigawa & Imamura (1977) in that antisera to the surface of oral epithelial cells do not equate with pemphigus antisera. It would appear possible that pemphigus antisera react or cross-react with antigens normally sequestered in the epithelial cell surface and released by cell damage.

ACKNOWLEDGMENT

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REFERENCES

- ABLIN, R.J. (1969) Immunopathologic studies of experimental pemphigus-like antibodies and bullous-like lesions. *Journal of Investigative Dermatology*, **53**, 463.
- BEUTNER, E.H., CHORZELSKI, T.P. & JORDON, R.E. (1970) *Autosensitization in Pemphigus and Bullous Pemphigoid*, p. 26. C.C. Thomas, Springfield, Illinois, U.S.A.
- BRIGDEN, W.D. & AMOS, H.E. (1975) The differential binding of antibody from the sera of patients with pemphigus and pemphigoid to isolated guinea-pig epidermal cells. *British Journal of Dermatology*, **93**, 425.
- CHORZELSKI, T.P., BICYSKO, W., DABROWSKI, J. & JARZABEK, M. (1968) Ultrastructural localization of pemphigus autoantibodies. *Journal of Investigative Dermatology*, **50**, 36.
- DOLBY, A.E. (1969) Effect of sera and peripheral blood lymphocytes upon oral epithelial tissue culture cells. *Immunology*, **17**, 709.
- DOLBY, A.E. (1972) Acute ulcerative gingivitis—immune complex. *Journal of Dental Research*, **51**, 1639.
- GROB, P.J. & INDERBITZIN, T.M. (1967a) Experimental production in rabbits of antiepithelial autoantibodies. *Journal of Investigative Dermatology*, **49**, 637.
- GROB, P.J. & INDERBITZIN, T.M. (1967b) Pemphigus antigen and blood group substances A and B. *Journal of Investigative Dermatology*, **49**, 285.
- MILNE, A. & DOLBY, A.E. (1972) A comparison of 2 methods of cell derivation for tissue culture. *Journal of Dental Research*, **50**, 1201.
- SELA, B., WANG, J.L. & EDELMAN, G.M. (1975) Antibodies reactive with cell surface carbohydrates. *Proceedings—National Academy of Sciences*, **72**, 1127.
- SINGER, S.J. & NICOLSON, G.L. (1972) The fluid mosaic model of the structure of cell membranes. *Science*, **175**, 720.
- TAKIGAWA, M. & IMAMURA, S. (1977) Experimental production of rabbit anti-guinea-pig epidermal cell sera. Comparison to pemphigus antibodies. *Journal of Investigative Dermatology*, **68**, 259.
- VAN LIS, J.M.J. & KALSBECK, G.L. (1975) The interaction of Concanavalin A and the surface coat of stratified squamous epithelium. *British Journal of Dermatology*, **92**, 27.

The relationship between immunological parameters and response to therapy in resistant oral candidosis

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SUMMARY

Eight patients with resistant oral candidal infection have undergone full immunological assessment. In four of these patients no significant abnormality of immune function was observed, and all responded to intensive antifungal therapy with nutritional supplements of iron and folic acid as required. The remaining four patients had abnormalities only of specific candida-related immune function and no detectable abnormality of general immune function. Surgical excision of affected areas of oral mucosa was found to be the most effective therapy in three of these four cases.

The immunological findings in these cases are compared with those observed in five patients with chronic mucocutaneous candidosis in whom both specific candida-related and non-specific immune incompetence were present.

Oral candidosis is a condition found chiefly in patients with predisposing local factors such as dentures, metabolic abnormalities such as diabetes, or in those on long term antibiotic or steroid therapy. On occasion, however, oral candida infection may affect apparently healthy individuals and in the majority of such instances a course of topical antifungal therapy using such agents as nystatin or amphotericin B is all that is required to effect a cure. This situation has recently been comprehensively reviewed (Cawson, 1976).

A more unfortunate group of individuals is that in which repeated courses of topical therapy are ineffective. These patients tend to become chronic attenders at departments of oral medicine or dermatology.

We report here eight such individuals all of whom were referred because of failure to respond to topical antifungal drugs.

MATERIAL AND METHODS

The age, sex and clinical variety of oral candidosis in each case are detailed in Table 1. All had had several trials of both topical nystatin and amphotericin B before assessment, and none was diabetic, or on long term antibiotic or steroid therapy. Nutritional status of these patients with reference to

serum iron and total iron binding capacity, whole blood folate and serum B₁₂ levels are also detailed in Table 1.

TABLE 1. Details of sex, age clinical type of candidosis, folate, B₁₂ and iron levels

Patient no.	Sex	Age	Clinical variety of infection	Corrected whole blood folate (mg/ml)	Se. B ₁₂ (pg/ml)	Hb. (Gm%)	Se.Fe. (μmol/l)	T.I.B.C. (μmol/l)
1	M	53	Hyperplastic	180	92	16.2G	12	63
2	M	80	Atrophic	48	293	10.9	22	66
3	M	22	Hyperplastic	74	568	11.7	5	63
4	F	34	Hyperplastic	63	353	13.4	13	54
5	F	26	Atrophic	190	139	14.0	9	87
6	F	54	Hyperplastic	72	298	13.1	9	78
7	F	46	Hyperplastic	58	590	15.0	27	60
8	M	43	Hyperplastic	168	309	15.9	16	84

* Details of *Candida*-related immunological investigations and those of general immune function are given in Table 2. All tests were performed by standard methods (references where appropriate). The novel aspect of this study was the quantification of specific *Candida* IgG antibody by a radio immunoassay developed by Nielsen, Parratt & White (1973) and evaluated by Cobb & Parratt (1977). This technique is more sensitive than fluorescent antibody or precipitin methods and is of proven value in the study of patients with candida infection (de Sousa *et al.*, 1976). The immunological assessment was directed mainly at humoral and cell-mediated aspects of lymphocyte function, but the candida killing and nitro blue tetrazolium tests were included as a measure of neutrophil function and were normal in all eight patients.

TABLE 2. Tests of immune function

Specific anti-candida tests	General immune assessment Differential white cell count Quantification of total serum IgG, A, M & E
Quantification of anti-candida IgG (Nielsen Parratt & White, 1973)	Lymphocyte transformation to PHA & PWM Total circulating E rosette forming lymphocytes (Jondal, Holmes & Wigsell, 1972)
Candida skin test (Bencard DHS)	Total circulating EAC rosette forming lymphocytes (Bianco, Patrick & Nussenweig, 1970)
Candida killing assay	Recall skin tests to PPD, & <i>T. rubrum</i> (Bencard), mumps (Eli Lilly) & SKSD (Lederle) NBT test

RESULTS

Results of tests of *Candida* related immunity prior to nutritional replacement or further antifungal therapy are detailed in Table 3. Of the eight patients, four (1-4) had no detectable abnormality of candida related immune function; specific anti-candida IgG levels were under 60 μg/ml on all occasions tested (normal range 0-60 μg/ml), positive *Candida* skin tests were observed and *Candida* mediated

lymphocyte transformation was present. The remaining four patients (5-8) had elevated anti-candida IgG (4/4), negative Candida skin tests (3/4) and negative candida mediated lymphocyte transformation (3/4).

Tests of general immune function yielded a normal profile in all eight patients. Serum immunoglobulin levels, lymphocyte transformation to phytohaemagglutinin and pokeweed mitogen, quantification of E and EAC rosette forming cells, and skin testing to recall antigens all yielded normal results.

TABLE 3. Results of tests of Candida related immunological activity

Patient no.	Candida antibody level (Normal range 0-60 µg/ml)	Candida skin test (Bencard DHS read at 24 & 48 h)	Candida mediated lymphocyte transformation
1	23	+	+
2	25	+	+
3	33	+	+
4	39	+	+
5	197	-	-
6	172	-	+
7	110	-	-
8	141	+	-

Response to further therapy

In view of the lack of any gross immunological defect in these patients, immune reconstitution was not required as a therapeutic procedure. Oral folic acid was therefore administered to Cases 2, 3, 4, 6 and 7 who had low initial serum folate levels, oral iron to Cases 1, 3, 4, 5, 6 and 8 and all patients were encouraged to persevere with topical nystatin and amphotericin B in rotation.

After 3-6 months all patients were reassessed. Four patients (1-4) had experienced complete clearance of their lesions during this period but four patients (5-8) had persistent lesions, despite correction of nutritional deficiencies. These patients were therefore considered candidates for surgery and three have responded well to excision of areas of hyperplastic oral mucosa colonized by Candida. Two of these have had cryotherapy and one conventional surgical excision.

Repeat assessment of Candida related immunological function after this period of intensive therapy and nutritional replacement showed no significant changes by comparison with those performed prior to therapy.

DISCUSSION

Comparison of the four patients responding to topical antifungal drugs, with additional folic acid and oral iron replacements where appropriate, with the four who required surgical intervention reveal clear differences in immunological reactions to *Candida albicans*. The four responders had normal levels of serum anti-candida IgG, and demonstrated by positive skin tests and lymphocyte transformation on exposure to Candida that cell mediated immune recognition was present. In contrast the four non-responders all had high serum anti-candida IgG, negative candida skin tests (3/4) and negative lymphocyte transformation (3/4). These patients would appear to demonstrate a state of specific immune imbalance to *Candida albicans* with excess antibody formation and poor cell mediated immunological activity. The possibility of a direct serum blocking factor was considered but repeated washing of the non-transforming lymphocytes prior to incubation did not result in Candida mediated lymphocyte stimulation.

The mechanism of this immune imbalance is not immediately clear, although several possible explanations are worthy of consideration; growth of *Candida albicans* on oral mucosa may result either in hyphal invasion of the stratum corneum leading to hyperplasia and hyperkeratosis or in degradation of the epithelium without invasion of hyphae, the atrophic form of infection. In either situation minor nutritional deficiencies may contribute to breakdown in the normal mucosal barrier, and subsequent immunological activation. In most individuals, recognition of *Candida* leads to humoral and cell mediated responses which can be of some value in effecting a cure providing that topical antifungal therapy is supported by correction of nutritional deficiencies. By contrast, in resistant individuals, excess production of *Candida* antibody may result in paralysis of *Candida* directed cell-mediated immunity (possibly a high zone tolerance effect), and therefore antifungal therapy and strengthening of the mucosal epithelium with nutritional replacements may be insufficient to effect a cure.

An alternative hypothesis is that in those individuals who did not respond to antifungal therapy and nutritional replacements, high levels of *Candida* antibody were present prior to this episode of infection as a result of a previous episode and that the immunological changes preceded clinical events. In two of the patients (6 and 7) monitoring of *Candida* antibodies which are slowly falling after successful surgery suggests that this is not the explanation but that the immunological changes are the sequelae of resistant infection rather than a primary cause of the situation.

A further possibility is that the four non-responding individuals may have had a delay in antibody response to the organism so that excessive amounts of antibody were produced but not until after the period of rapid multiplication of *Candida albicans*. Thus the growth of microorganisms was uncontrolled, and excessive antibody production inhibited an appropriate cell mediated immune response. This of course assumes an effective role for circulating antibody in the control of *Candida albicans*, a subject which will be the basis of future publications. It is known that opsonisation of the organism leads to its efficient removal by polymorphonuclear leucocytes (Wilkinson, personal communication), and this process should at least partially control multiplication of the organism.

A comparison of the eight patients in this study with those with chronic mucocutaneous candidosis of either the diffuse or familial variety shows clear differences in immunological abnormalities. Such patients have very much higher levels of anti-candida IgG (Fig. 1) and in our experience abnormalities of general immunological function are commonly found in addition to specific *Candida*-related deficiencies.

Nutritional deficiencies and their relationship to candida infection are an interesting and topical problem (Higgs & Wells, 1972). In our series three of the four responders (2, 3 and 4) and two of the non responders (6 and 7) had initial low levels of folate, which rose in all cases to normal levels with treatment. Similarly Cases 1 and 3 in the responding group had low serum iron levels as had Cases 5, 6 and 8 in the non-responding group. We have not seen in this series a case completely refractory to oral iron administration as has been reported by Higgs (1973) and it seems likely that such resistance to oral iron therapy is a specific feature of the mucous membrane in chronic mucocutaneous candidosis.

Thus all of the patients in our study required folic acid, oral iron or both as supplements and although all showed laboratory evidence of absorption and response to this therapy, only four showed clinical benefit. It is therefore suggested that while correction of nutritional deficiency will not in all situations result in response to antifungal therapy, replacements should be given before resort to more time consuming and traumatic methods of management. Certainly our four responders had all had adequate topical antifungal therapy prior to nutritional replacement and had not responded clinically. Simultaneous antifungal therapy and nutritional replacement was, however, successful.

It is of note that of our five patients with low folate levels four had the hyperplastic variant of oral candidosis. A significant relationship between this variant of candidosis and nutritional deficiency

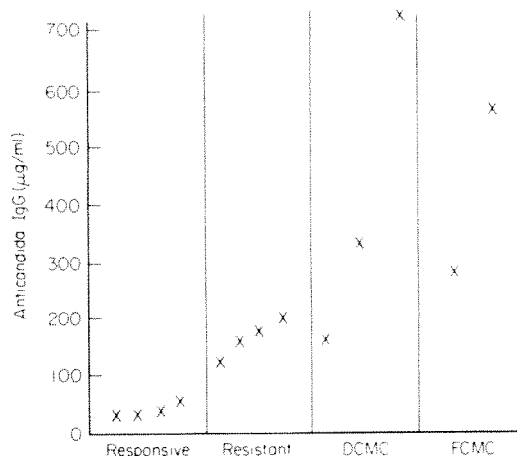


FIGURE 1. Comparison of anti-candida IgG levels in 4 patients responsive to nutritional replacements and antifungal therapy, 4 patients resistant to this treatment, three patients with diffuse chronic mucocutaneous candidosis (DCMC) and two with familial chronic mucocutaneous candidosis (FCMC). Normal levels of anti-candida IgG are from 0 to 60 µg/ml.

has been reported (Jenkins *et al.*, 1977). Similarly a positive relationship between the hyperplastic variant and low serum iron levels has been established and 5 of our 6 patients with low serum iron did have this clinical variant.

A relationship between clinical variants of candidal infection and immunological changes has been observed by Lehner who reports that in patients with the 'acute pseudomembranous' variant, significant titres of anti-candida IgG, A and M can be observed by immunofluorescence techniques *prior* to infection, whereas patients with the atrophic variety have no such elevation prior to infection (Lehner & Ward, 1970). Unfortunately, it was not possible to ascertain anti-candida antibody levels in our group of patients prior to clinical presentation, and only by large population studies, or screening of 'at risk' individuals can this observation be confirmed.

The general problem of nutritional status and immunological competence is one which is currently the cause of much interest (Bhaskaram & Reddy, 1975). In addition to the improved immune function after correction of depleted iron stores reported by Dagg *et al.* (1966), Axelrod has reported that pyridoxine deficiency may result in both defective antibody formation and cell mediated responses (Axelrod & Trakatellis, 1964). The fact, however, that the only immunological abnormalities observed in the series we have reported were candida-related strongly suggests that nutritional factors were not greatly affecting immune function.

Our observations would suggest that in patients with oral candida infection which is apparently resistant to topical antifungal therapy, assessment of folate and serum iron levels, together with immunological parameters may be of value in deciding on further appropriate management. If Candida antibody levels are within normal limits, and cell mediated immune responses to candida are detectable, then persevering with topical antifungal treatment and correction of nutritional deficiencies is an appropriate line of treatment. If, however, Candida antibody levels are raised and cell mediated reactions are not detectable, surgery is probably the treatment of choice.

REFERENCES

- AXELROD, A.E. & TRAKATELLIS, A.C. (1964) Relationship of Pyridoxine to immunological phenomena. *Vitamins and Hormones*, **22**, 591.

- BHASKRAM, C. & REDDY, V. (1975) Cell mediated immunity in iron and vitamin deficient children. *British Medical Journal*, **iii**, 522.
- BIANCO, C., PATRICK, R. & NUSSENSWEIG, V. (1970) Population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. *Journal of Experimental Medicine*, **132**, 702.
- CAWSON, R.A. (1976) Infections of the oral mucous membrane. In: *Scientific Foundations of Dentistry* (ed. by B. Cohen & I.R.H. Kramer), Ch. 41, pp. 484-497. Heinemann Medical, London.
- COBB, S. & PARRATT, D. (1977) The determination of antibody levels to *Candida albicans* in healthy and hospitalized adults using a radioimmunoassay. *Clinical and Experimental Dermatology*, in press.
- DAGG, J.H., JACKSON, J.M., CURRY, B. & GOLDBERG, A. (1966) Cytochrome oxidase in latent iron deficiency (sideropenia). *British Journal of Haematology*, **12**, 331.
- HIGGS, J.M. (1973) Chronic mucocutaneous candidiasis: iron deficiency and the effects of iron therapy. *Proceedings of the Royal Society of Medicine*, **66**, 802.
- HIGGS, J.M. & WELLS, R.S. (1972) Chronic mucocutaneous candidiasis. Associated abnormalities of iron metabolism. *British Journal of Dermatology*, **86**, Suppl. 8, 88.
- JENKINS, W.M.M., MACFARLANE, T.W., FERGUSON, M.M. & MASON, D.K. (1977) Nutritional deficiency in oral candidosis. *International Journal of Oral Surgery*, **6**, 204.
- JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface merities on human T and B lymphocytes. *Journal of Experimental Medicine*, **136**, 207.
- KIRKPATRICK, C.H., RICH, R.R. & BENNETT, J.E. (1971) Chronic mucocutaneous candidiasis, model building in cellular immunity. *Annals of Internal Medicine*, **74**, 955.
- LEHNER, T. (1972) Cell mediated immune responses in oral disease: A review. *Journal of Oral Pathology*, **1**, 39.
- LEHNER, T. & WARD, R.G. (1970) Iatrogenic oral candidosis. *British Journal of Dermatology*, **83**, 161.
- NIELSEN, K.H., PARRATT, D. & WHITE, R.G. (1973) Quantitation of antibody to particulate antigen using a radiolabelled immunoglobulin reagent. *Journal of Immunological Methods*, **3**, 130.
- DE SOUSA, M., COCHRAN, R., MACKIE, R., PARRATT, D. & ARALE-CHAWES, M. (1976) Chronic mucocutaneous candidiasis treated with transfer factor. *British Journal of Dermatology*, **94**, 79.

Erythropoietic protoporphyria: hepatic cirrhosis

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SUMMARY

Observations on liver pathology and function were made on 12 patients with erythropoietic protoporphyria (EPP). Needle liver biopsies in 3 of 11 patients showed mild portal or periportal fibrosis without evidence of abnormal liver function. One patient (Case 12) died from hepatic cirrhosis and failure at the age of 11 years, the youngest yet reported. He was not suspected of pending liver disease until 3 months before death. The quantity of protoporphyrin present in his liver was approximately 5.75% of total liver weight. Fatal liver disease in EPP has occurred in 13 patients, 7 male and 6 female, in which the mean age of death was 38 years. The pathology of the liver in EPP is characteristic and is described.

The dermatological signs and symptoms of photosensitivity in erythropoietic protoporphyria (EPP) are well documented (Rimington *et al.*, 1967), and this condition can be considered relatively common and usually benign. EPP was first described by Magnus *et al.* (1961) and in the subsequent 6 years the incidence was slightly greater in males—64 males and 36 females.

The purpose of this brief report and review is to re-emphasize that not only has the liver in EPP a characteristic pathology including deposition of protoporphyrin, but also periportal fibrosis or even severe cirrhosis may be present more commonly than is suspected. The liver was examined in 12 patients; fibrosis was noted in 3 of 11 patients who had no evidence of abnormal liver function (Table 1). However, another patient (Case 12) had severe cirrhosis and hepatic failure, and death occurred at the age of 11 years. He was not suspected of having liver disease until 3 months before death. Fatal liver disease in EPP has thus occurred in 13 patients, 7 male and 6 female, and is later summarized.

PATIENTS AND METHODS

The age, sex and relevant liver pathology of 12 patients with EPP are summarized in Table 1. Cases 1–5 have been described previously by Cripps & Scheuer (1965). These patients and patients 6–11 had no evidence of liver dysfunction. Case 12, an 11-year-old boy, with severe photosensitivity

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TABLE 1. Liver biopsies from 12 patients with EPP summarizing sex, age, porphyrin, liver function and liver pathology

Patient	Sex	Age	Liver		Protoporphyrin		Stool ($\mu\text{g/g}$ dry wt)	Liver ($\mu\text{g/g}$ wet wt)
			Fibrosis	Function	Pigment	RBC ($\mu\text{g } \%$)		
1	F	18	+	N	+	1108	132	
2	F	22		N	+	1086	36	7.4
3	M	45		N	o	556	12	
4	F	15	+	N	+	716	3	
5	F	21		N	+	745	16	
6	M	21		N	o	473	83	
7	F	16		N	+	1410	91	
8	M	11		N	o	1200	136	
9	M	18		N	+	765	199	
10	F	23	+	N	+	1250	475	
11	M	8		N	o	540	248	
12	M	11	+++	+	++	8700	1580	57.500
Normals			o	N	o	< 52	< 40	o

developed slight jaundice and a mild retention of sulphobromophthalein (13.5%) but a normal serum transaminase 3 months before admission; at that time he was not known to have EPP. When examined he weighed 25 kg, was jaundiced, and the liver felt hard but was not tender and was palpable to the level of the anterior-superior iliac crest. Ascites was present and the spleen was palpable two fingers below the left costal margin (Fig. 1). Hepatic function deteriorated with the serum bilirubin level rising from 3.2 to 17.4 mg/dl, the serum alkaline phosphatase level to 73.5 iu and the serum transaminase level from 282 to 669 iu. He subsequently died from massive bleeding from oesophageal varices. Two weeks before death he had a perforated peptic ulcer which was repaired following abdominal laparotomy, and a liver biopsy was also obtained.

Histopathology of the Liver

Percutaneous liver biopsies were obtained from all 12 cases described in Table 1 and by open laparotomy and later at autopsy from Case 12. Unfixed frozen sections as well as sections of formalin-fixed paraffin embedded material were examined. Histological methods used included haematoxylin

FIGURE 1. Erythropoietic protoporphyria: Case 12. Male aged 11. Liver palpable to anterior superior iliac crest, portal hypertension and ascites.

FIGURE 2. Portal and periportal fibrosis (silver impregnation) Case 4 (similar features to Cases 1 and 10) ($\times 80$).

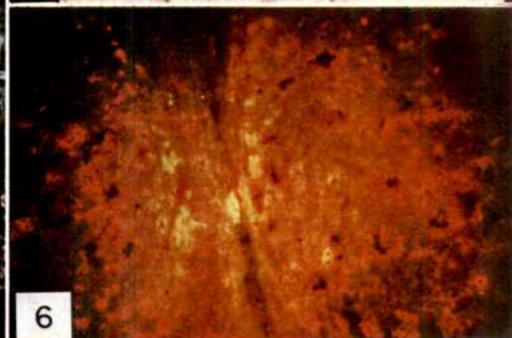
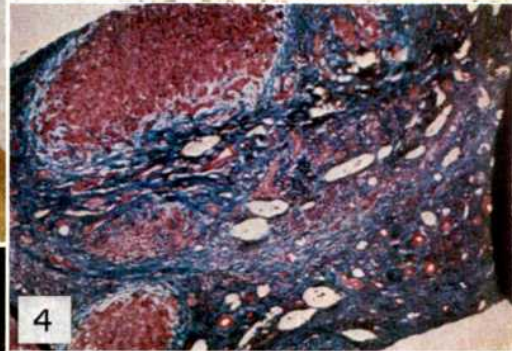
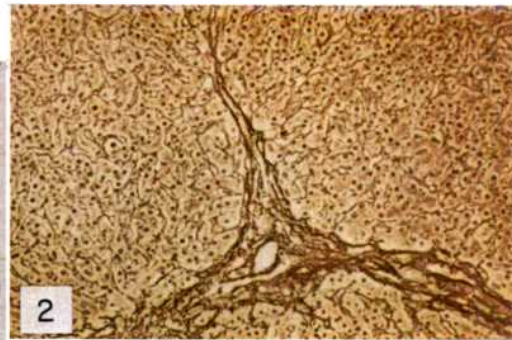
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FIGURE 3. Liver (Case 12). Gross appearance: Enlarged nodular, fibrotic, dark brown with protoporphyrin content of 5.75 mg/g wet weight.

FIGURE 4. Liver biopsy (Case 12). Hyperplastic hepatocellular nodules are separated by dense fibrous scars (Masson trichrome stain, $\times 150$).

FIGURE 5. Electronmicroscopy of a pigment-containing Kupffer cell. A localized round cluster of crystals seen free in the cytoplasm and close to the nucleus ($\times 26,400$).

FIGURE 6. Hepatic protoporphyrin fluorescence of brown pigment in nodules separated by non-fluorescing fibrous scar ($\times 320$).



and eosin, Perl stain for iron, silver impregnation for reticulin and also Masson's trichrome stain for Case 12. Examination was performed by fluorescence and polarizing light microscopy. The liver of Case 12 was examined by electron microscopy, for which small fragments of liver tissue were fixed in 4% cacodylate buffered glutaraldehyde, post fixed in 1% osmium tetroxide, dehydrated through graded alcohols, embedded in epon araldite, cut on an LKB ultramicrotome and viewed in a Hitachi Hs-8 electron microscope.

Porphyrin determinations

Faecal coproporphyrin, uroporphyrin and protoporphyrin; urinary coproporphyrin and uroporphyrin were determined by the method reported by Rimington *et al.* (1961). Erythrocyte, plasma and liver protoporphyrin determinations were based on a method by Rimington *et al.* (1963).

RESULTS

Porphyrins (Table 1)

Erythrocyte protoporphyrin of Case 12, who died of liver failure, was high (8650–9650 $\mu\text{g}/100\text{ ml}$), in contrast to the mean of 850 $\mu\text{g}/100\text{ ml}$ of the more typical patients with EPP (Cripps (1974) and as illustrated in Table 1). The estimated protoporphyrin of the liver was exceedingly high (57.5 mg/g wet weight), the highest recorded. In addition, the stool protoporphyrin of Case 12 was also high (1530 $\mu\text{g}/\text{g}$ dry weight), which is also higher than the usual mean of 304 $\mu\text{g}/\text{g}$ dry weight.

Histopathology (Table 1)

Cases 1–11. Lobular structure appeared intact in all 11 liver needle biopsies. Portal and peri-portal fibrosis was observed in Cases 1, 4 and 10 (Fig. 2). Brown pigment deposits (protoporphyrin) were found in biopsy specimens from Cases 1, 2, 4, 5, 7, 9 and 10 in hepatocytes and in Kupffer cells and mostly concentrated in and around bile ducts. The brown pigment examined by polarized microscopy had distinctive birefringence with prominent maltese cross patterns (Klatskin & Bloomer, 1974) and showed characteristic protoporphyrin autofluorescence under fluorescence microscopy. In addition a rapidly fading diffuse porphyrin fluorescence was noted in the cytoplasm of liver parenchymal cells which on light microscopy did not appear to be abnormal.

Case 12. The liver was greatly enlarged (1700 g), dark brownish-black (Fig. 3), firm and distorted by numerous hyperplastic nodules which measured up to 5 cm in diameter. Nodules were separated by dense fibrous scars, typical of post-necrotic cirrhosis. Microscopically on H & E stain the liver parenchymal cells varied greatly in size, many of them appearing swollen. Abundant, finely dispersed and more clumped brown pigment was observed in hepatocytes and in Kupffer cells, showing the same appearances as described above. Masson's trichrome stain (Fig. 4) indicated dense fibrous tissue separating hepatocellular nodules.

On electron microscopy distinctive crystalline structures were noted in aggregates and more diffusely in Kupffer cells and in hepatocytes. They were composed of short, straight or slightly curved rod-like structures with tapered ends (Fig. 5) and similar to those reported by Wolff *et al.* (1975).

DISCUSSION

Most livers with EPP would appear to have a distinctive pathology—deposits of granular brown pigment that do not stain for bile or iron, which fluoresce red grossly and microscopically and which are identified as protoporphyrin (Fig. 6). In addition, the protoporphyrin observed in routine H & E

formalin fixed sections has distinctive birefringent properties (Klatskin & Bloomer, 1974). The protoporphyrin is found in hepatocytes and in Kupffer cells, mostly concentrated initially in and around bile ducts. Electron microscopy of the liver in EPP (Mascaro *et al.*, 1973; Bloomer *et al.*, 1974; Wolff *et al.*, 1974) has shown the presence of needle like inclusions or crystals. Deposition of the relatively insoluble protoporphyrin in crystalline form may initiate fibrosis as was observed mildly in 3 of 11 patients (Table 1) and illustrated in Fig. 2, who had no clinical or laboratory evidence of liver damage, and very severely in Case 12 where the protoporphyrin content was so great that it accounted for approximately 5.75% of the total liver weight. The quantity of hepatic protoporphyrin is compared with that in other patients with EPP who have died from liver failure.

Fatal liver disease in EPP has occurred in 7 males and 6 females with a mean age of 38 years. The first was reported by Barnes *et al.* (1968), a male aged 42 with liver protoporphyrin 4900 μg per gram wet weight; Donaldson *et al.* (1971) reported 2 males aged 56 and 58, the younger of which had liver protoporphyrin of 208 μg ; Schmidt & Stitch (1971) reported a female aged 40; Iwanov *et al.* (1973) a male aged 42; Scott *et al.* (1973) a female aged 43; Thomson *et al.* (1973) two sisters aged 31; Meffert *et al.* (1974) a female aged 40; Bloomer *et al.* (1975) a female aged 32, liver protoporphyrin of 1787 μg ; Meyer *et al.* (1975) a male aged 16; MacDonald & Nicholson (1976) a male age 54; compared with our Case 12 (1977) male aged 11 with 57,500 μg .

Two other patients with EPP who have died are not included above; Donaldson *et al.* (1971), in addition to reporting two brothers who died, referred to a personal communication of death occurring in an 8-year-old boy with EPP who died of portal hypertension but no autopsy was performed. Bloomer *et al.* (1975) also referred to an unreported case of Stringer's, a 40-year-old female who died of EPP.

An important aspect of hepatic disease in EPP is that clinical and laboratory evidence of hepatic involvement may not become apparent until the terminal illness. This is typified in one of the sisters reported by Bloomer *et al.* (1975), and by patient 12, both of whom died of hepatic failure 3 months after development of jaundice. The only laboratory abnormality noted was slight sulphobromophthalein retention and borderline increase in serum transaminase.

A progressively increasing protoporphyrin in the erythrocytes or its occurrence in the urine may also be a sign of impending liver damage. In Case 12, terminal erythrocyte protoporphyrin values were 8650–9650 $\mu\text{g}/100\text{ ml}$. Donaldson *et al.* (1971) reported 6000 $\mu\text{g}/100\text{ ml}$, Bloomer *et al.* (1975) values up to 9374 $\mu\text{g}/100\text{ ml}$ and MacDonald & Nicholson (1972) up to 6020 $\mu\text{g}/100\text{ ml}$.

The occurrence of gallstones in EPP at an age younger than usual has been reported by Magnus *et al.* (1961), Haeger-Aronson (1963), Cripps & Scheuer (1965), Lynch & Miedler (1965), Prentice & Goldberg (1967), Stern *et al.* (1969), and in one of the sisters reported by Thomson *et al.* (1973). Kniffen (1970) suggested oral cholestyramine for removal of protoporphyrin in porphyraemia and this treatment might have been helpful in our Case 12 if the diagnosis had been made earlier.

CONCLUSIONS

Liver biopsies from 3 of 11 patients with EPP, who had no clinical or laboratory evidence of liver dysfunction, showed portal or peri-portal fibrosis (example illustrated in Fig. 3). However, fatal liver disease has occurred in only 13 cases which suggests the possibility that these could be examples of a distinctive type of EPP with protoporphyrin hepatopathy. Clinical and laboratory signs of hepatic damage may develop insidiously with initially normal or near normal liver function tests. The seriousness of hepatic complications suggests that patients with EPP should have regular assessment of hepatic function.

REFERENCES

- BARNES, H.D., HURWORTH, E. & MILLAR, J.H.D. (1968) Erythropoietic porphyrin hepatitis. *Journal of Clinical Pathology*, **21**, 157.
- BLOOMER, J.R., PHILLIPS, M.J., DAVIDSON, D.L. & KLATSKIN, G. (1975) Hepatic disease in erythropoietic protoporphyria. *American Journal of Medicine*, **58**, 869.
- CHARLES, R., BEIDLER, G. & JOHNSON, B.L. (1974) Erythropoietic protoporphyria—a new role for the fibroblasts. *Archives of Pathology*, **97**, 79.
- CRIPPS, D.J. (1974) Porphyrin patterns in porphyria. *Wisconsin Medical Journal*, **73**, 103.
- CRIPPS, D.J. & PETERS, H.A. (1967) Fluorescing erythrocytes and porphyrin screening tests on urine, stool and blood. *Archives of Dermatology*, **96**, 712.
- CRIPPS, D.J. & SCHEUER, P.J. (1965) Hepatobiliary changes in erythropoietic protoporphyria. *Archives of Pathology*, **80**, 500.
- DONALDSON, E.M., MCCALL, A.J., MAGNUS, I.A., SIMPSON, J.R., CALDWELL, R.A. & HARGREAVES, T. (1971) Erythropoietic protoporphyria: Two deaths from hepatic cirrhosis. *British Journal of Dermatology*, **84**, 14.
- HAEGER-ARONSEN, B. (1963) Erythropoietic protoporphyria. *American Journal of Medicine*, **35**, 450.
- IWANOV, E., TASCHEV, T., KRASDEV, L. & BARIKOV, N. (1972) Leberschädigung bei Protoporphria erythropoetica. *Dermatologische Monatsschrift*, **158**, 806.
- KLATSKIN, G. & BLOOMER, V.R. (1974) Birefringence of hepatic deposits in erythropoietic protoporphyria. *Gastroenterology*, **67**, 294.
- KNIFFEN, J.C. (1970) Protoporphyrin removal in intrahepatic porphyrastasis. *Gastroenterology*, **58**, 1027.
- LYNCH, P.J. & MEIDLER, L.J. (1965) Erythropoietic protoporphyria. *Archives of Dermatology*, **92**, 351.
- MACDONALD, D.M. & NICHOLSON, D.C. (1976) Erythropoietic protoporphyria: Hepatic implications. *British Journal of Dermatology*, **95**, 157.
- MAGNUS, I.A., JARRETT, A., PRANKERD, T.A.J. & RIMINGTON, C. (1961) Erythropoietic protoporphyria: A new porphyria syndrome with solar urticaria due to protoporphyrinaemia. *Lancet*, **ii**, 448.
- MASCARO, J.M., PINOL, A., GALLY-MASCARO, C., BRUGUERA, M. & ESQUERDI, J.E. (1973) Ultraestructura de hígado en la protoporphyria erythropoietica. *Medicina Cutanea Ibero-Latino-Americana*, **7**, 119.
- MATILLA, A. & MOLLAND, E.A. (1974) A light and electromicroscopy study of the liver in a case of erythropoietic protoporphyria and in griseofulvin-induced porphyria in mice. *Journal of Clinical Pathology*, **698**, 709.
- MEFFERT, H., BARTHELMES, H., SCHMIDT, B. & SONNICHSEN, H. (1974) Leberbeteiligung bei erythropoietischer Protophyrie. *Dermatologische Monatsschrift*, **160**, 748.
- MEYER, U.A., FAH, P., KNOBLAUCH, M. & FRICK, P. (1975) Protoporphyrin hepatopathy: Rare complications of erythropoietic protoporphyria or separate disease? Abstract *Porphyrins in Human Disease*. International porphyrin meeting, Friburg, Germany. p. 57. University of Marburg.
- PRENTICE, R.T.W. & GOLDBERG, A. (1967) Erythropoietic protoporphyria. *British Journal of Dermatology*, **79**, 682.
- RIMINGTON, C. (1961) *Quantitative determinations of porphobilinogen and porphyrins in the urine and faeces*. Broad sheet 36. Blackwell Scientific Publications, Oxford.
- RIMINGTON, C., MORGAN, P.N., NICHOLLS, K., EVERALL, J.D. & DAVIS, R.R. (1963) Griseofulvin administration and porphyrin metabolism. *Lancet*, **ii**, 318.
- RIMINGTON, C., MAGNUS, I.A., RYAN, E.A. & CRIPPS, D.J. (1967) Porphyria and photosensitivity. *Quarterly Journal of Medicine*, **36**, 29.
- SCHMIDT, D. & STITCH, W. (1971) Erythropoietische Protoporphyrinurie mit Porphyrinurie: Untersuchungen zur Frage der Leberbeteiligung bei erythropoietischer Protoporphyrinurie. *Blut*, **22**, 202.
- SCOTT, A.J., ANSFORD, A.J., WEBSTER, R.H. & STRINGER, H.C.W. (1973) Erythropoietic protoporphyria with features of a sideroblastic anemia terminating in liver failure. *American Journal of Medicine*, **54**, 251.
- STERN, W.K., GORDON, M. & URBACH, F. (1970) Erythropoietic protoporphyria: An unusual variant reacting abnormally in the erythema spectrum. *Archives of Dermatology*, **99**, 730.
- THOMPSON, R.P.H., MOLLARD, E.A., NICHOLSON, D.C. & GRAY, C.H. (1973) Erythropoietic protoporphyria and cirrhosis in sisters. *Gut*, **14**, 934.
- WOLFF, K., WOLFF-SCHREINER, E. & GSCHNAIT, F. (1975) Liver inclusions in erythropoietic protoporphyria. *European Journal of Clinical Investigation*, **5**, 21.

The effects on epidermal DNA synthesis of the butyrate esters of clobetasone and clobetasol, and the propionate ester of clobetasol

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SUMMARY

An animal model for evaluating the effects of drugs on epidermal DNA synthesis has been used to determine the time of onset, period of maximum activity and duration of effect of two topically applied proprietary glucocorticosteroids, clobetasone butyrate and clobetasol propionate. Both drugs produced maximum inhibition of epidermal DNA synthesis by 24 h after application. Clobetasol propionate exhibited a significantly earlier onset ($4\frac{1}{2}$ h) and longer duration (36 h) of activity than clobetasone butyrate (6 and 24 h respectively). A systemic effect was not observed at any time after the application of clobetasone butyrate but was observed for up to 24 h after the application of clobetasol propionate.

Clobetasol butyrate was also assayed in this system and was found to be as potent as clobetasol propionate. It is concluded that the alcoholic, as opposed to the ketonic, grouping in the 11 positions of the steroid ring profoundly alters the potential of this molecule for systemic activity.

A hairless mouse model for evaluating the effects of drugs on epidermal cell division has been described previously (du Vivier *et al.*, 1976). The modification and use of the model to study the effects of topically applied glucocorticosteroid formulations have also been described (du Vivier, Marshall & Brookes, 1978). Further studies to determine the time of onset, period of maximum antimitotic activity and duration of both local and systemic effects of two steroids, clobetasone butyrate (Molivate) and clobetasol propionate (Dermovate), with a view to designing more effective therapeutic schedules for these drugs, have now been completed.

Important differences had already been observed between these two steroids, in particular the fact that while clobetasone butyrate had a marked local effect but no systemic effect, clobetasol propionate was very potent in both areas (du Vivier, Marshall & Brookes, 1977). It was therefore decided to undertake further study of this unexpected lack of effect of clobetasone butyrate by investigating clobetasol butyrate.

MATERIALS AND METHODS

Animals

Hairless mice (strain A2hr), random bred, 2–4 months old and of either sex were used. Animals were dosed in three groups of six; two of the groups with a cream formulation of the drug under investigation and one group with Cream E45 or Molivate cream base as control.

Formulations

(i) 0.05% clobetasone butyrate cream (Molivate cream: Glaxo); (ii) 0.05% clobetasol propionate cream (Dermovate cream: Glaxo); (iii) 0.05% clobetasol butyrate in Molivate cream base (Glaxo); (iv) Control—white soft paraffin 14.5%, light liquid paraffin 11.6%, anhydrous lanolin 1% in a specially formulated emulsion base (Cream E45: Boots); and (v) Base for clobetasone butyrate cream (Molivate cream base: Glaxo).

PROCEDURE

0.03 cm³ of the test drug formulation was applied, and spread over 3 cm² of the anterior dorsal skin (hereafter referred to as the neck) with a seeker, for 60 s. The posterior half of the dorsal surface of the animal (hereafter referred to as the back) was left untreated. All animals were given 25 μ Ci (methyl-³H) thymidine, specific activity 5 Ci/mmol (The Radiochemical Centre, Amersham), subcutaneously in the right thigh 1 h before being killed by ether inhalation. The neck and back skin samples, with at least 2 cm of unsampled skin left between them (to avoid inclusion of treated skin in the back sample), were removed. The back skin was dissected well away from the site of injection of tritiated thymidine. The epidermis was then separated from the dermis by heat treatment (25 s at 57°C) and stored at –20°C until required for analysis.

To determine its specific activity, the DNA was extracted from lysates of the epidermal samples by column chromatography using hydroxylapatite. The DNA solutions were quantified spectrophotometrically and their radioactivities determined by scintillation counting in the manner described previously (du Vivier, Marshall & Brookes, 1977).

Activity/time profiles

Eight separate experiments were conducted, the animals being killed at 3, 4½, 6, 12, 24, 36, 48 and 72 h after application of the test formulation.

Structure/activity relationship

The different steroids were applied once only (as cream formulations) and the animals were killed 24 h after application of the test formulation.

RESULTS

These are recorded in Tables 1, 2 and 3 and they are expressed as mean disintegrations per minute (d.p.m.) per μ g DNA $\pm$ standard error (s.e.). Table 1 shows the effect of the test formulations on the synthesis of DNA in the treated neck area (designated local effect) and Table 2 the effect on the synthesis of DNA in the untreated back area (designated systemic effect). Table 3 shows the differing effects of the butyrate esters of clobetasone and clobetasol on epidermal DNA synthesis 24 h after a single application.

TABLE 1. Local effects of clobetasone butyrate and clobetasol propionate on epidermal DNA synthesis (Mean* d.p.m./ μ g DNA $\pm$ s.e.)

Time (h)	Cream E45	Clobetasone butyrate cream	<i>P</i>	Clobetasol propionate cream	<i>P</i>
3	105 $\pm$ 15	113 $\pm$ 10	N.S.	112 $\pm$ 18	N.S.
4½	144 $\pm$ 19	138 $\pm$ 20	N.S.	85 $\pm$ 13	<0.05
6	124 $\pm$ 12	80 $\pm$ 6	<0.01	53 $\pm$ 5	<0.001
12	386 $\pm$ 29	71 $\pm$ 9	<0.001	50 $\pm$ 7	<0.001
24	227 $\pm$ 25	31 $\pm$ 3	<0.001	26 $\pm$ 3	<0.001
36	218 $\pm$ 43	208 $\pm$ 26	N.S.	82 $\pm$ 22	<0.01
48	161 $\pm$ 29	255 $\pm$ 32	<0.01	123 $\pm$ 29	<0.05
72	191 $\pm$ 14	217 $\pm$ 18	N.S.	129 $\pm$ 19	<0.05

* n = 6. N.S. (Not significant), $P > 0.05$.

Although clobetasol propionate had a significantly earlier onset (4½ h) and longer duration (36 h) of activity than clobetasone butyrate (6 and 24 h respectively) both drugs exhibited maximum inhibition of DNA synthesis by 24 h (Table 1). A significant increase in DNA synthesis was seen at 48 h after the application of the latter, but not after that of the former at any time under investigation.

Clobetasol propionate had a significant systemic effect at 6 h which was maximal by 24 h. At 36 h DNA synthesis had returned to the Control level. Clobetasone butyrate had no effect on DNA synthesis in the untreated back skin at any time (Table 2).

TABLE 2. Systemic effects of clobetasone butyrate and clobetasol propionate on epidermal DNA synthesis (Mean* d.p.m./ μ g DNA $\pm$ s.e.)

Time (h)	Cream E45	Clobetasone butyrate cream	<i>P</i>	Clobetasol propionate cream	<i>P</i>
3	122 $\pm$ 21	111 $\pm$ 34	N.S.	130 $\pm$ 30	N.S.
4½	120 $\pm$ 12	113 $\pm$ 16	N.S.	87 $\pm$ 11	N.S.
6	138 $\pm$ 16	152 $\pm$ 17	N.S.	99 $\pm$ 5	<0.05
12	154 $\pm$ 18	186 $\pm$ 44	N.S.	51 $\pm$ 5	<0.001
24	163 $\pm$ 18	129 $\pm$ 15	N.S.	37 $\pm$ 4	<0.001
36	195 $\pm$ 31	232 $\pm$ 24	N.S.	295 $\pm$ 56	N.S.
48	91 $\pm$ 15	132 $\pm$ 19	N.S.	97 $\pm$ 27	N.S.
72	173 $\pm$ 14	171 $\pm$ 15	N.S.	141 $\pm$ 10	N.S.

* n = 6. N.S. (Not significant), $P > 0.05$.

Twenty-four hours after a single application of clobetasone butyrate or clobetasol butyrate there was a significant decrease in DNA synthesis locally in the treated neck area (Table 3). The effect of the latter, however, was significantly greater than that of the former. Similarly, in the back area, clobetasol butyrate had a highly significant effect on DNA synthesis, whereas clobetasone butyrate had no such action.

From Table 1 it can be seen that Cream E45 increased local DNA synthesis at 12, 24 and 36 h.

TABLE 3. The local and systemic effects of the butyrate esters of clobetasone and clobetasol on epidermal DNA synthesis 24 h after a single application (Mean* d.p.m./ μ g DNA $\pm$ s.e.)

Drug	Neck		Back	
		<i>P</i>		<i>P</i>
(a) Vehicle alone	121 $\pm$ 15	—	124 $\pm$ 18	—
(b) Clobetasone butyrate	31 $\pm$ 4	<0.001	121 $\pm$ 31	N.S.
(c) Clobetasol butyrate	9 $\pm$ 2	<0.001	46 $\pm$ 5	<0.01
(c) < (b)		<0.001		<0.05

*n = 6. N.S. (Not significant), $P > 0.05$.

DISCUSSION

Glucocorticosteroids are the most frequently prescribed topical drugs, yet little work is done to relate therapeutic effects and application frequencies. This practice would seem to be of prime importance since tachyphylaxis has been demonstrated to their vasoconstrictor (du Vivier & Stoughton, 1975) and antimitotic properties (du Vivier, 1976). Such therapeutic scheduling is already an integral part of cancer chemotherapy and it therefore seems appropriate to determine the length of action of topically applied glucocorticosteroids with regard to their effects on epidermal cell division so that there can be a more logical approach to their prescription.

Our findings with the very potent steroid, clobetasol propionate, indicate that this is a long-acting steroid and that one application per day should be sufficient to maintain suppression of DNA synthesis and, at the same time, minimize the occurrence of tachyphylaxis and systemic effects. The findings with the moderately potent steroid, clobetasone butyrate, suggest a shorter duration of activity and hence a different dosage schedule. The correlation between our findings and clinical practice is evidenced by the different recommended dosages for these two steroids (see ABPI Data Sheet Compendium, 1977). Such individualized dosage schedules for each steroid formulation may decrease unwanted side effects.

It is of interest that a significant increase in local DNA synthesis is seen 48 h after the application of clobetasone butyrate. No such local increase in DNA synthesis is seen at any time point following the application of clobetasol propionate, although this may indicate that time points later than 72 h should be tested. Such an increase may account for the 'rebound' effect so often seen when treating psoriasis with topical steroids (Comaish, 1965). From this and other results (Marshall, 1977) it is speculated that this increase in synthesis is related to the rate of loss of activity, i.e. the faster the fall-off, the greater the rebound.

The effect of Cream E45 in stimulating local DNA synthesis was unexpected and the choice of this formulation was unfortunate since the control neck skin was stimulated, whereas the back area remained unaffected. However, the significance of the findings remains the same whether the results are compared directly with; (a) those following the application of Cream E45, (b) those of the other steroid at that particular time, (c) those of an earlier timing for the steroid being investigated, or, (d) the control values for the clobetasone butyrate base. However, these observations emphasize how important the choice of vehicle is, and how sensitive the assay system is in demonstrating such a vehicle effect on DNA synthesis.

The lack of systemic toxicity of clobetasone butyrate is of particular interest, as had been noted before (du Vivier, Marshall & Brookes, 1977). At no time during the nine experiments (fifty-four

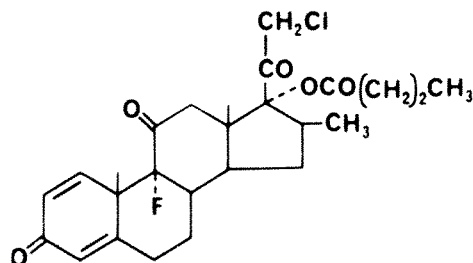


FIGURE 1. Clobetasone butyrate.

tested animals) was a systemic effect noted with clobetasone butyrate, despite a highly significant local activity. The formula of this steroid is depicted in Fig. 1. The unusual feature of this molecule is the presence of a ketone group in position 11 on the steroid ring. It would appear that there is no other proprietary topical glucocorticosteroid with a ketone group in this position—all others have a hydroxyl group. It is well known that cortisone acetate, which has an 11-ketone group, has no effect when used topically whereas hydrocortisone, with an 11-hydroxyl group, is effective.

It is proposed that the 9-fluoro, 16-methyl, 17-butyrate and 21-chloro substituents so increase the potency and/or lipid solubility of this steroid that, either they counteract the deleterious effect of the 11-ketone grouping, or they impart sufficient activity to any small amounts of the 11 β -hydroxyl steroid prepared *in situ* by metabolic conversion.

To test the importance of the ketone group, clobetasol butyrate, which differs from clobetasone butyrate only in having a hydroxyl group at the 11 position, was tested in the system. In vasoconstriction studies it was predicted to have similar potency to clobetasol propionate (Wilson, 1976). In the hairless mouse model our results show conclusively that, like clobetasol propionate, clobetasol butyrate has a profound effect both locally and systemically.

This work confirms the suggestion made by Elks (1976) that topical and systemic activity need not be linked and it may be possible to synthesize steroids with very potent local antimitotic activity but without systemic side effects.

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REFERENCES

- COMAISH, S. (1965) Ingram method of treating psoriasis. *Archives of Dermatology*, **92**, 56.
- DU VIVIER, A. (1976) Tachyphylaxis to topically applied steroids. *Archives of Dermatology*, **112**, 1245.
- DU VIVIER, A., BIBLE, R., MIKURIYA, R. & STOUGHTON, R.B. (1976) An animal model for screening drugs for antipsoriatic properties using hydroxyapatite to rapidly isolate DNA from the epidermis. *British Journal of Dermatology*, **94**, 1.
- DU VIVIER, A., MARSHALL, R.C. & BROOKES, L.G. (1978) An animal model for evaluating the local and systemic effects of topically applied corticosteroids on epidermal DNA synthesis. *British Journal of Dermatology*, **98**, 209.
- DU VIVIER, A. & STOUGHTON, R.B. (1975) Tachyphylaxis to the action of topically applied glucocorticosteroids. *Archives of Dermatology*, **111**, 581.
- ELKS, J. (1976) Steroid structure and activity. *British Journal of Dermatology*, **94**, suppl. 12, 3.
- MARSHALL, R.C. (1977) Personal observations and unpublished data.
- WILSON, L. (1976) Personal communication.

Brief Communication

Evidence for defective T cell function in patients with alopecia areata

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The role of immune factors in the pathogenesis of alopecia areata (AA) is still the object of considerable theoretical and experimental debate. Our interest in the problem was recently stimulated by the serendipitous finding of a low percentage of circulating T lymphocytes in two subjects with AA. This finding prompted us to assess the percentage of peripheral blood lymphocytes forming rosettes with sheep erythrocytes (E-rosettes: T cells), as well as of lymphocytes forming rosettes with sheep erythrocytes coated with IgM antibody and complement (EAC rosettes: B cells) and of lymphocytes forming rosettes with human erythrocytes coated with anti-CD IgG antibodies (EA-rosettes: 'L' or 'null' cells) in 25 subjects affected by AA and in an equal number of age- and sex-matched healthy controls. In both groups the age ranged from 15 to 52 years and the male/female ratio was 7/18. All subjects were in good general health and were taking no drugs at the time of the study.

The mean percentage of circulating T cells in subjects with AA (43.16 ± 2.56 s.d.) was much lower than that of controls (61.3 ± 0.53 s.d.); Student's 't'-test performed after angular transformation of the data showed that the difference between the two means was statistically significant ($P < 0.005$). On the contrary, in the subjects with AA the mean percentages of B cells (12.4 ± 0.6 s.d.) as well as of L cells (18.14 ± 1.8 s.d.) were not significantly different from those in controls (B cells: 11.2 ± 0.8 s.d.; L cells: 10.24 ± 0.9 s.d.).

Of the 25 tested patients, only 7 had percentages of circulating T cells above 50 (the lower limit of normal range in our laboratory); 3 of the latter 7 patients were in remission, whereas all remaining patients had percentages of T lymphocytes below 50 and were in the active phase of the disease.

Some groups have reported a high frequency of autoantibodies in AA (Cunliffe *et al.*, 1969; Main *et al.*, 1975) and T lymphocytes are known to be involved in the control of autoantibody 'triggering' (Lachmann, 1975). Further studies are in course to clarify the clinical and pathogenetic significance of these findings and speculations.

REFERENCES

- CUNLIFFE, W., HALL, R., STEVENSON, C.J. & WEIGHTMAN, D. (1969) Alopecia areata, thyroid disease and autoimmunity. *British Journal of Dermatology*, **81**, 877.
MAIN, R.A., ROBBIE, R.B., GRAY, E.S., DONALD, D.E. & HORNE, C.H.N. (1975) Smooth muscle antibodies and alopecia areata. *British Journal of Dermatology*, **92**, 389.
LACHMANN, P.J. (1975) Autoallergy. In: *Clinical Aspects of Immunology* (Ed. by P.G.H. Gell, R.A. Coombs and P.J. Lachmann), 3rd edn, p. 859. Blackwell Scientific Publications, Oxford.

Review Article

Aerobic cutaneous coryneforms: recent taxonomic findings

The golden age of medical microbiology, when the major pathogens were identified and conquered by advances in chemotherapy, has given way to more sustained research concerned with less virulent but none-the-less important microbial invasions of the human body. Many of the organisms regarded as invaders are also members of the normal flora. Normally they may be helpful in occupying a niche which might be taken by more virulent organisms interacting with these to prevent colonization (Selwyn, 1975). But on occasion they may, due to predisposing factors, multiply abnormally causing damage to the host.

Koch's postulates are difficult to demonstrate in such a situation and we are forced to reconsider the term 'pathogenicity'. Microbial disease can sometimes be ascribed to the formation of a specific toxin, such as that produced by *Corynebacterium diphtheriae* and here the term 'pathogen' may be used with confidence. However, many subacute conditions yield only non-toxin producing normal members of the human microflora. Williams (1973) has explored the 'benefit and mischief' that may result from the presence of the normal body flora in the right, or wrong, place.

The current trend, especially amongst microbiologists interested in the skin, is now away from the classical concepts of 'pathogenic' and 'commensal' members of the flora and towards regarding skin as a complex ecosystem. Changes in the balance of organisms or in host/microbe interactions can result in shifts from commensal to pathogenic status. Kligman (1965), describing skin from the germ's eye view, called it a varied world of jungles, swamps and deserts. A wide variety of microbes inhabit this world and to describe them requires a comprehensive taxonomy. Surprisingly, adequate taxonomies for the skin microflora are of very recent origin; indeed for some groups, as will become apparent, they do not yet exist, but obviously the investigation of a complex ecosystem without an adequate taxonomy can only lead to confusion and misconception.

Probably the least studied of skin micro-organisms are the aerobic diphtheroids. This term has been used since the early days of microbiology to signify organisms morphologically similar to the diphtheria bacillus. Bacteria of this type are, however, among the most ubiquitous in the environment, having been found in soil and air, fresh and salt water, from the tropics to the Polar regions. They include many animal and plant pathogens. Most bacteriologists now refer to these organisms as 'coryneforms', using the word in the same sense as the term 'coliform' is used for Gram negative rods. Only the medical world still retains the term diphtheroid. Although these terms are far from satisfactory, there are no alternatives at the present.

The history of the coryneforms in the medical field began with Loeffler's (1884) discovery of '*Bacillus diphtheriae*' in diphtheritic lesions. Confusion soon arose, however, when apparently indistinguishable bacilli were obtained from a variety of other lesions and also from healthy people. Interest in these 'pseudodiphtheria bacilli' became intense and many diseases, including leprosy, general paralysis of the insane, typhus and Hodgkin's disease were attributed to them. This chaotic situation was reviewed by Ebersson (1918) in the first serious attempt at classification, principally based on descriptions of cellular and colonial morphology and sufficiently detailed to enable present day coryneform isolates to be recognized as the types seen by Ebersson. Eleven of the 23 species

described in his work were isolated from human skin and mucous membranes and all were given specific names. Andrewes *et al.* (1923) reduced this to three species, two of which, *Corynebacterium xerosis* and *C. pseudodiphtheriticum* are still officially listed (International Committee for Systematic Bacteriology, 1976).

Once the distinction between *C. diphtheriae* and other morphologically similar organisms had been established, interest in the latter waned. Barritt (1933), after completing large numbers of sugar tests and animal experiments, was forced to admit that 'apart from *C. diphtheriae*, no constant relationship between source, biochemical, morphological or other characters of diphtheroids could be found'.

The advent of antibiotics pushed interest in the 'diphtheroids' even further into the background and it was not until after the publication in 1965 of Mary J. Marples' book *The Ecology of Human Skin* that interest in the normal flora was renewed.

It is now apparent that the normal flora of the skin is in a state of flux; a dynamic equilibrium exists, although the composition varies between individuals and between sites on the same individual. This balance between the different members of the skin flora is flexible (Kloos & Musselwhite, 1975; Bibel, Lovell & Smiljanic, 1976) but major changes in microbial equilibrium may have unpleasant consequences for the individual. Aerobic coryneforms play a large part in the ecosystem and may make up more than 50% of the total microbial flora. Intertriginous regions carry the greatest numbers of organisms.

Coryneform skin diseases

Three common skin conditions appear to be related to an over-abundance of aerobic coryneforms on the skin. These are erythrasma, trichomycosis axillaris and pitted keratolysis (Sarkany, Taplin & Blank, 1961; Crissey, Rebel & Laskas, 1952; Taplin & Zaias, 1963). The first two are most common in the humid areas, the axillae, toeweb and groin, the third is characteristic of soggy feet. The principal predisposing factor therefore appears to be a high local humidity, but other host factors such as diabetes may also play a part (Savin, 1974). The application of occlusion to a site may also result in an over-abundance of coryneforms accompanied by maceration.

Attempts to identify individual diphtheroids as causative for one or more of these conditions have been inconclusive and a wide variety of coryneform biotypes have been isolated from skin lesions (Shehadeh & Kligman, 1963; McBride, Freeman & Knox, 1968; Savin, Somerville & Noble, 1970).

The specific agent of erythrasma was thought to have been found when Sarkany, Taplin & Blank (1961) described *C. minutissimum*. This species was said to be identifiable by its ability to produce a coral red fluorescence when grown in suitably enriched media and viewed under a Wood's lamp. The attraction of this idea was that the bacteria were isolated from the scaling lesions of erythrasma which fluoresced similarly. The transfer of organisms onto healthy skin could cause a fresh area of scaling and fluorescence. However the authors needed to occlude the skin in order to reproduce the lesion, and, as has been stated, this could lead to an increase in all coryneforms and not just the newly deposited strain.

Subsequently, Somerville (1973) found that the property of fluorescence was possessed by a large number of coryneform biotypes and could also be demonstrated in micrococci. Although this does not necessarily invalidate the species *C. minutissimum*, it is now clear that criteria other than porphyrin production should be used to define this group. In fact, the ability to produce and excrete excess porphyrins is widely distributed among bacteria (Lascelles, 1961), and may be stimulated by an increase in the supply of porphyrin precursors (especially 5-aminolaevulinic acid). An increase in lactate also stimulates porphyrin production by forcing an increase in respiratory chain cytochrome synthesis (Doss & Phillipp-Dormston, 1973). Lactate is probably the principal carbon source in sweat, and may be abundant in occluded areas. It is also possible that the medium recommended for

the demonstration of fluorescence, which contains fetal calf serum, is high in porphyrin precursors.

In supplying exogenous 5-aminolaevulinic acid to 31 skin coryneforms of varied biochemical characteristics in a simple medium, I have found that 21 strains were capable of rapid porphyrin production (unpublished observations).

Many other properties of aerobic skin coryneforms, for example lipophilia, lipolysis and keratinolysis have attracted attention as taxonomic characters. Though important ecological factors, they have not so far proved to be very useful from the taxonomic viewpoint. One commonly used feature which appeared to have taxonomic value is nitrate reduction. Evans (1968) and subsequent authors consistently found higher proportions of nitrate reducing coryneforms in the anterior nares than on other sites. The ecological significance of this finding is, however, rather hard to discern.

The presence of nitrate in nasal secretions does not appear to have been established and the anterior nares must be one of the most aerated sites of the body. However, nitrate reductase activity in bacteria is suppressed by a high pO_2 . The conclusion must be, therefore, that dissimilatory reduction is not at work and that the enzyme may be cofunctional with other essential reductases (Arden & Barksdale, 1976): another typical example of the difficulty in relating ecological observations to bacterial characteristics.

It will be clear from the foregoing discussion that many questions of aetiology and ecology remain unanswered. Rapid strides are being made in understanding the distribution and function of cutaneous cocci and the *Propionibacterium acnes* complex but similar progress cannot be made with the aerobic coryneforms until an adequate taxonomy is available.

Taxonomic studies

It has always been assumed that aerobic coryneforms are members of the genus *Corynebacterium*, though this has never been satisfactorily proved. Using this assumption, several workers have described schemes for classifying aerobic cutaneous coryneforms (Evans, 1968; Marples, 1969; Smith, 1969; Somerville, 1973; Kasprowicz, Heczko & Kucharczyk, 1974), but without clear cut results. Dichotomous schemes such as that of Somerville have the advantage that all isolates can be fitted into a named group, but give undue weight to the first character in the scheme. Tabloid schemes, though giving equal weight to characters, can only accommodate all the biotypes isolated when a very large number of tests are performed.

The work of Cummins and his associates (1956, 1958, 1962) established that the clearest marker for the genus *Corynebacterium* sensu stricto is the presence in the cell wall of both *meso* 2,6 diamino-pimelic acid (DAP) and arabino-galactan. The first of these components, part of the peptidoglycan, is unique to certain bacterial cell walls. Polysaccharides containing one to four neutral sugars are commonly present in large amounts in the cell walls of coryneforms isolated from all habitats and clear differences occur in the patterns obtained for several genera.

The limited number of diamino acids and sugars in cell walls make the identification of these components one of the most useful tools available to taxonomists, and because they are unique or present in high concentration, analysis for cell wall components in dried whole cells can be carried out with reasonable confidence avoiding the need to prepare clean cell walls (Keddie & Cure, 1977).

In an attempt to confirm the position of aerobic skin coryneforms as members of the genus *Corynebacterium*, I have (Pitcher, 1977) analysed 800 isolates from a variety of body sites and individuals for cell wall components. The pattern which emerged showed that only about 60% of the isolates could be assigned to the genus *Corynebacterium*, but that a second group, representing about 20% of the isolates, possessed cell walls containing *meso*-DAP and galactose but lacking arabinose. This combination is found in *Brevibacterium linens*, a coryneform common in cheese. Other cell wall patterns found were based on *LL*-DAP with arabinose (about 2%), diaminobutyric acid (DAB)

with rhamnose (about 2%) and the remainder in which neither DAP or DAB could be detected. The latter possessed a wide variety of sugars.

Strains conforming to the *Corynebacterium* pattern were more frequently isolated from sites which are the least accessible to the environment (Table 1). In contrast, strains not possessing DAP or DAB were very much more commonly isolated from exposed sites. Only four diamino acids have been detected in cell walls so that the absence of DAP and DAB in these strains infers that the diamino acid present is either lysine or ornithine which are indicative of *Arthrobacter* and other genera commonly found in air, soil and water. These could not be detected from whole cell hydrolysates since they occur in intracellular proteins.

The *Brevibacterium* type of isolates which must now be recognized as members of the resident human skin flora showed little site preference.

The strains which possessed LL-DAP with arabinose but no galactose, were usually isolated from toeweb and must be considered as residents since this unusual combination has not been reported from any other source than human skin. These strains form a fairly homogeneous group and because of their unusual cell walls may represent an entirely new genus (Pitcher, 1976). In wall composition they are intermediate between the anaerobic *Propionibacterium* spp which have LL-DAP and galactose but not arabinose (Johnson & Cummins, 1972) and the aerobic *Corynebacterium* spp which have meso, DAP, arabinose and galactose.

The strains in which DAB and rhamnose were detected were uncommon and no firm conclusions could be drawn concerning their role as members of the resident flora, though it was curious that a disproportionate number came from the axillae of a small panel of industrial workers. Since coryneforms possessing DAB in their cell walls are principally found on plants or in water, this may be due to occupational contamination. Other sites on members of the panel were not examined.

Ecological significance of Brevibacterium isolates

Sharpe, Law & Phillips (1976), in describing a new group of coryneforms isolated from dairy products, postulated human skin as a possible source on the grounds that their strains, unlike other dairy coryneforms, were salt tolerant and grew luxuriantly at 37°C. These white *brevibacteria*, like the orange cheese coryneform *Brevibacterium linens*, were found to be capable of producing the gas methanethiol (CH_3SH) from L-methionine. Methanethiol is a major component of the flavour and aroma of ripe cheese (Manning, 1974) and is detected by the method of Ruiz-Herrera & Starkey (1969). Subsequent studies (Sharpe *et al.*, 1977) showed that white *brevibacteria* from skin were closely related to the cheese isolates and were also potent producers of CH_3SH .

Pitcher (unpublished observations) has been unable to detect CH_3SH production in skin isolates conforming to *Corynebacterium* but found that about half of skin *Brevibacterium* isolates could produce this gas. Since this property may have ecological significance and preliminary studies indicate that considerable differences in other biochemical characteristics, colonial form and appearance also exist, it is proposed that CH_3SH producing isolates from skin be designated *Brevibacterium* I, and isolates incapable of producing CH_3SH but possessing the same cell wall pattern be designated *Brevibacterium* II.

The only substrate utilized by *Brevibacterium* I in the production of CH_3SH appears to be methionine. A study of the availability of this amino acid on the skin surface may therefore contribute to our knowledge of body odour. More recent unpublished work (Pitcher & Youssef) indicates that these bacteria are more commonly isolated from the toeweb of persons suffering from tinea pedis due to *Trichophyton* spp of fungi than in those in which dermatophytes cannot be demonstrated. Preliminary investigations show that, unlike the majority of *Corynebacterium* strains, *Brevibacterium* I strains are resistant to penicillin. Bearing in mind the demonstration by Youssef *et al.* (1976) of the

TABLE 1. Cell wall composition of coryneforms from human skin

Identity	Total isolates	% with cell walls components					DAP & DAB not detected
		<i>meso</i> -DAP Arabinose	<i>meso</i> -DAP Galactose	LL-DAP Arabinose	DAB Rhamnose		
		<i>Corynebacterium</i> spp	<i>Brevibacterium</i> spp	?	?		
Total exposed sites (forehead, ear, forearm, hand, leg)	304	43	25	1	1	30	
Interriginous sites (axilla, perineum, toewebs)	496	67	20	2	2	8	

DAP = 1,6-diaminopimelic acid; DAB = 1,4-diaminobutyric acid.

production of penicillin-like antibiotics by dermatophyte fungi, a little of the complexity of the skin ecosystem can be unravelled. It seems possible that *Brevibacterium* I strains are responsible for the assertion by Kligman, Leyden & McGinley (1976) that the production of sogginess, maceration, itching and malodour as a sequel to dermatophytosis simplex in tinea pedis is due to 'diphtheroids'. The dermatophyte fungi may even be responsible for making methionine available by keratinolysis, although the *Brevibacterium* I strains are themselves proteolytic.

Computer taxonomy of coryneforms

The technique of numerical taxonomy is rapidly gaining support for studying the inter-relationships between bacteria. Its rationale is that, in the absence of any convenient and rapid method of determining natural genetic relationships among bacteria at the level of DNA codons, the nearest approach is to list as many expressions of bacterial genotype as possible and compare the results obtained for different strains (phenotypic analysis). Since such large numbers of observable characters and strains are involved, this requires the use of a computer. It also removes the problem of weighting inherent in dichotomous schemes.

Pitcher (in preparation) has applied numerical taxonomic techniques to 69 aerobic skin coryneforms representing all the cell wall types previously found using 97 test characters. These were compared with a selection of 59 reference strains from coryneform and other genera covering a wide spectrum of sources and habitats. Skin coryneforms, in spite of differences in their cell wall composition, remained surprisingly homogeneous as a group compared with coryneforms from other habitats and allied genera (e.g. *Mycobacterium* and *Nocardia*) from which they were clearly separated.

Other skin isolates with non DAP cell walls associated with coryneforms from environmental sources. There was a basic division of skin isolates into three groups. One of these, *Brevibacterium* I phenon was clearly separate from the rest of the skin strains. The remainder were broadly split into two phenon on the basis of their ability to oxidize glucose. Reference strains of *C. pseudodiphtheriticum* fell into the first of these (non-oxidative), though a close relationship with wild strains in the same phenon was not seen. The second (oxidative) phenon contained reference strains of *C. xerosis* and *C. minutissimum* and pathogenic species such as *C. ulcerans*, *C. pseudotuberculosis* and *C. renale*.

The *Brevibacterium* II group formed a discrete cluster within the non-oxidative phenon, though neither they nor the *Brevibacterium* I phenon showed any obvious relationships to reference strains.

Analysis for lipid corynomycolic acids by the acid methanolysis technique of Minnikin, Alshamaony & Goodfellow (1975) was carried out on all the strains subjected to numerical taxonomy and confirmed the separation of aerobic skin coryneforms into *Corynebacterium* and *Brevibacterium*. Corynomycolic acids were only associated with *Corynebacterium* cell wall components; mycolic acids serve to distinguish between *Mycobacterium*, *Nocardia* and *Corynebacterium* species, all of which possess meso-DAP and arabinose.

In summary we can say that the application of modern taxonomic techniques such as cell wall analysis and numerical taxonomy confirm that we have still much to learn about the microflora of our own skins. Although human skin strains are more closely related to each other than to strains from other habitats, there is a substantial diversity. At least one genus has not been described previously from skin or elsewhere. A second genus, *Brevibacterium*, has been recognized as resident although *Corynebacterium* sensu stricto are the most common inhabitants of human skin being less common in other habitats. About half of the *Brevibacterium* strains are able to produce the gas methanethiol from methionine; this gas is probably involved in malodour of the feet perhaps following dermatophyte infection.

It is hoped that further application of modern taxonomic techniques to the aerobic coryneform

flora of man, and the construction of simple schemes of classification, will reveal more of the ecology of these interesting inhabitants of our skins.

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REFERENCES

- ANDREWES, F.W., BULLOCK, W., DOUGLAS, S.R., DREGER, G., GARDNER, A.D., FILDES, P., LEDINGHAM, J.C.G. & WOLF, C.G.L. (1923) In: *Diphtheria: Its Bacteriology, Pathology and Immunology*, pp. 370-426. H.M.S.O. London.
- ARDEN, S.B. & BARKSDALE, L. (1976) Nitrate reductase activity in lysogenic and non-lysogenic strains of *Corynebacterium diphtheriae* and related species. *International Journal of Systematic Bacteriology*, **26**, 66.
- BARRITT, M.M. (1933) A group of aberrant members of the genus *Corynebacterium* isolated from the human pharynx. *Journal of Pathology*, **XXXVI**, 369.
- BIBEL, D.J., LOVELL, D.J. & SMILJANIC, R.J. (1976) Effects of occlusion upon population dynamics of skin bacteria. *British Journal of Dermatology*, **95**, 607.
- COWAN, S.T. (1968) In: *A Dictionary of Microbial Taxonomic Usage*. Oliver and Boyd, Edinburgh.
- CRISSEY, J.T., REBELL, G.C. & LASKAS, J.L. (1952) Studies on the causative organism of trichomycosis axillaris. *Journal of Investigative Dermatology*, **19**, 187.
- CUMMINS, C.S. (1962) Chemical composition and antigenic structure of cell walls of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Actinomyces* and *Arthrobacter*. *Journal of General Microbiology*, **28**, 35.
- CUMMINS, C.S. & HARRIS, M.M. (1956) The chemical composition of the cell wall in some gram positive bacteria and its possible value as a taxonomic character. *Journal of General Microbiology*, **14**, 583.
- CUMMINS, C.S. & HARRIS, H. (1958) Studies on the cell wall composition and taxonomy of actinomycetales and related groups. *Journal of General Microbiology*, **18**, 173.
- DOSS, M. & PHILLIP-DORMSTON, W.K. (1973) Regulatory link between lactate dehydrogenase and the biosynthesis of porphyrin and heme in micro-organisms. *Enzyme*, **16**, 28.
- EBERSON, F. (1918) A bacteriological study of the diphtheroid organisms with special reference to Hodgkin's disease. *Journal of Infectious Diseases*, **23**, 1.
- EVANS, N.M. (1968) The classification of aerobic diphtheroids from human skin. *British Journal of Dermatology*, **80**, 81.
- INTERNATIONAL COMMITTEE FOR SYSTEMATIC BACTERIOLOGY (1976) Approved lists of bacterial names. *International Journal of Systematic Bacteriology*, **26**, 563.
- JOHNSON, J.L. & CUMMINS, C.S. (1972) Cell wall composition and deoxyribonucleic acid similarities among the anaerobic Coryneforms, classical Propionibacteria and strains of *Arachnia propionica*. *Journal of Bacteriology*, **109**, 1047.
- KASPROWICZ, A., HECZKO, P.B. & KUCHARCZYK, J. (1974) Proba Klasyfikacji Maczogowcow Skornyck. *Medycyna Doswiadczalna i Mikrobiologia*, **26**, 267.
- KEDDIE, R.M. & CURE, G.L. (1977) The cell wall composition and distribution of free mycolic acids in named strains of Coryneform bacteria and in isolates from natural sources. *Journal of Applied Bacteriology*, **42**, 229.
- KLIGMAN, A.M. (1965) The bacteriology of normal skin. In: *Skin Bacteria and their Role in Infection* (Ed. by H.I. Maibach and G. Hildick-Smith). McGraw Hill, New York.
- KLIGMAN, A.M., LEYDEN, J.J. & MCGINLEY, K.J. (1976) Bacteriology. *Journal of Investigative Dermatology*, **67**, 160.
- KLOOS, W.E. & MUSSELWHITE, M.S. (1975) Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Applied Microbiology*, **30**, 381.

- LASCELLES, J. (1961) Synthesis of tetrapyrroles by micro-organisms. *Journal of Biological Chemistry*, **41**, 417.
- LOEFFLER, F. (1884) Untersuchung über Bedeutung der Mikroorganismen für die Entstehung der Diphtherie beim Menschen, bei der Taube und beim Kalbe. *Arbeiten aus den Kaiserlichen Gesundheitsamt (Berlin)*, **2**, 421.
- MCBRIDE, M.E., FREEMAN, R.G. & KNOX, J.M. (1968) The bacteriology of trichomycosis axillaris. *British Journal of Dermatology*, **80**, 509.
- MANNING, D.J. (1974) Sulphur compounds in relation to Cheddar cheese flavour. *Journal of Dairy Research*, **41**, 81.
- MARPLES, M.J. (1965) *The Ecology of Human Skin*. Charles C. Thomas, Springfield, Illinois.
- MARPLES, R.R. (1965) The effect of hydration on the bacterial flora of the skin. In: *Skin Bacteria and their Role in Infection* (ed. by H.I. Maibach and G. Hildick-Smith), pp. 33-41. McGraw Hill, New York.
- MARPLES, R.R. (1969) Diphtheroids on normal human skin. *British Journal of Dermatology*, **81**, Suppl. 1, 47.
- MINNIKIN, D.E., ALSHAMAONY, L. & GOODFELLOW, M. (1975) Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *Journal of General Microbiology*, **88**, 200.
- PITCHER, D.G. (1976) Arabinose with LL-diaminopimelic acid in the cell wall of an aerobic coryneform organism isolated from human skin. *Journal of General Microbiology*, **94**, 225.
- PITCHER, D.G. (1977) Rapid identification of cell wall components as a guide to the classification of aerobic coryneform bacteria from human skin. *Journal of Medical Microbiology*, **10**, 439.
- RUIZ-HERRERA, J. & STARKEY, R.L. (1969) Dissimilation of methionine by a demethiolase of *Aspergillus* species. *Journal of Bacteriology*, **99**, 764.
- SARKANY, I., TAPLIN, D. & BLANK, H. (1961) The etiology and treatment of erythrasma. *Journal of Investigative Dermatology*, **37**, 283.
- SAVIN, J.A. (1974) Bacterial infections in diabetes mellitus. *British Journal of Dermatology*, **91**, 481.
- SAVIN, J.A., SOMERVILLE, D.A. & NOBLE, W.C. (1970) The bacterial flora of trichomycosis axillaris. *Journal of Medical Microbiology*, **3**, 352.
- SELWYN, S. (1975) Natural antibiosis among skin bacteria as a primary defence against infection. *British Journal of Dermatology*, **93**, 487.
- SHARPE, M.E., LAW, B.A. & PHILLIPS, B.A. (1976) Coryneform bacteria producing methanethiol. *Journal of General Microbiology*, **94**, 430.
- SHARPE, M.E., LAW, B.A., PHILLIPS, B.A. & PITCHER, D.G. (1977) Methanethiol production by Coryneform bacteria: strains from dairy and human skin sources and *Brevibacterium linens*. *Journal of General Microbiology*, **101**, 345.
- SHEHADEH, N.M. & KLIGMAN, A.M. (1963) The effect of topical antibacterial agents on the bacterial flora of the axilla. *Journal of Investigative Dermatology*, **40**, 61.
- SMITH, R.F. (1969) Characterisation of human cutaneous lipophilic diphtheroids. *Journal of General Microbiology*, **55**, 433.
- SOMERVILLE, D.A. (1973) A taxonomic scheme for aerobic diphtheroids from human skin. *Journal of Medical Microbiology*, **6**, 215.
- TAPLIN, D. & ZAIAS, N. (1963) The etiology of pitted keratolysis. *International Congress of Dermatology*, **1**, 593.
- WILLIAMS, R.E.O. (1973) Benefit and mischief from commensal bacteria. *Journal of Clinical Pathology*, **26**, 811.
- YOUSSEF, N., WYBORN, C., HOLT, G., NOBLE, W.C. & CLAYTON, Y.M. (1976) Production of antibiotics by dermatophyte fungi. *Journal of Medical Microbiology*, **9**, viii.

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News and Notices

The Royal College of Physicians of Edinburgh

The following dermatologists have recently been elected to the Fellowship of the Royal College of Physicians, Edinburgh:

Dr R.S.Chapman
Dr N.G.Fraser
Dr D.Gill

Dr M.I.Keir
Dr A.N.Okoro
Dr E.Wyatt

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometer(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

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1. Med J Aust. (1973), Special Suppl. 1, 10



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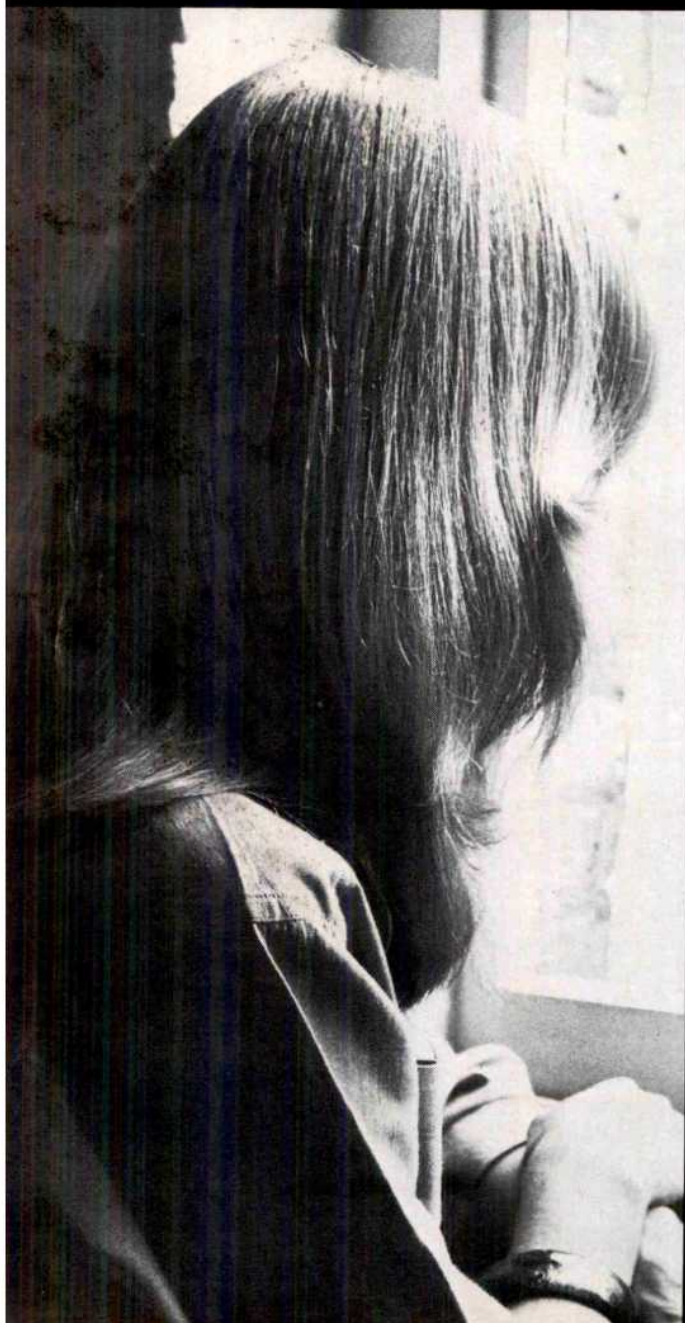
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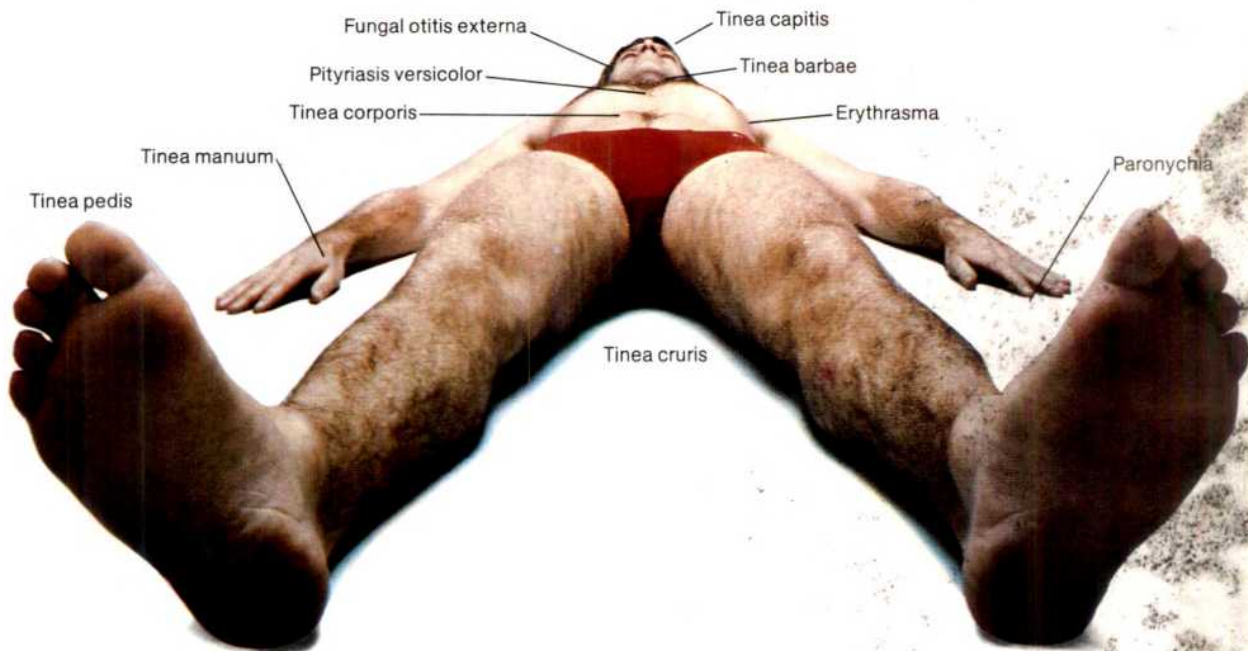
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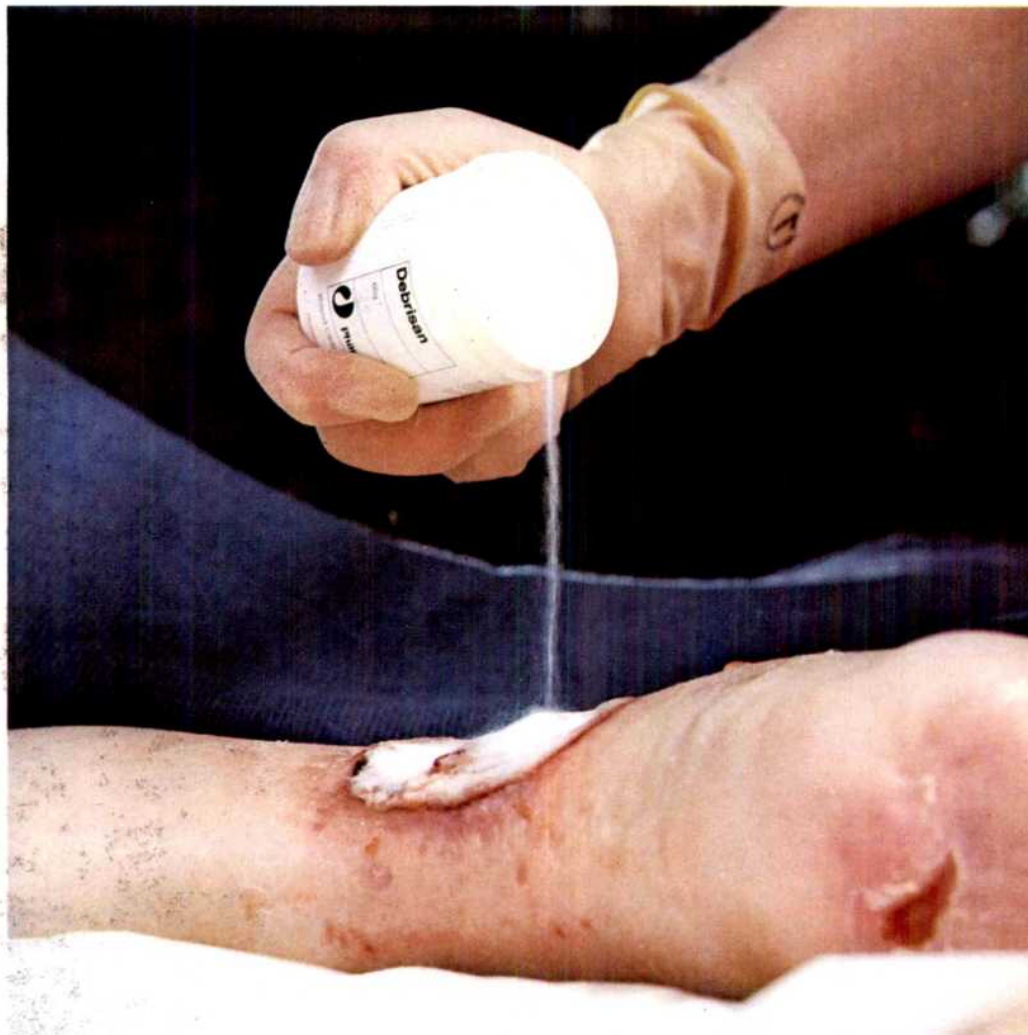
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'A New Principle for the Cleansing of Infected Wounds', Jacobsson, S., Rothman, U., Arturson, G. et al (1976) Scand. J. Plast. Reconstr. Surg. 10, 65-72

This paper reviews the mode of action, properties and clinical experience with Debrisan. It was found that a layer of Debrisan on a wound drew up exudate containing bacteria, degradation products, proteins and prostaglandin. In-vitro studies showed that bacteria were transported through the layer as it took up fluid. At saturation as few as 8.2% of the total bacteria in the layer were in the quarter of the layer in contact with the contaminated surface.

Clinically, Debrisan was found to halve the time for the preparation of wounds for grafting compared with the standard treatment in the hospital. In leg ulcers a good effect was noted in 70% of patients, shown as decreased inflammation and secretion, appearance of granulation tissue and relief of pain. In other wounds a 74% success rate was noted. A case is described of the healing and grafting of a leg ulcer treated with Debrisan which had previously resisted treatment for 23 years.

'The Effect of Dextranomer (Debrisan) on Hand Burns', Paavola, P. and Sundell, B. (1976). Ann. Chir. et Gynaec. 65, 313-317

17 II and III degree burned hands were treated with Debrisan. All hands recovered full mobility. Pain rapidly decreased and no crust formed, allowing daily physiotherapy. The hands remained free of infection; no side effects occurred. Grafting of three III degree burns was performed after two weeks' treatment without complications. Debrisan treatment produced better healing and was less time consuming than the previous routine treatment.

'Dextran Polymer Particles (Debrisan) in the Treatment of Penile Ulcers', Lassus, A. et al (1977) Acta Dermatovenereol (Stockholm) 57, 361-363

In 25 patients with ulcers due to herpes, dequalinium or unknown aetiology but of non-venereal origin, Debrisan treatment healed the majority in a week and all patients in four weeks. Relief of pain was most notable in this type of case which is frequently difficult to heal.



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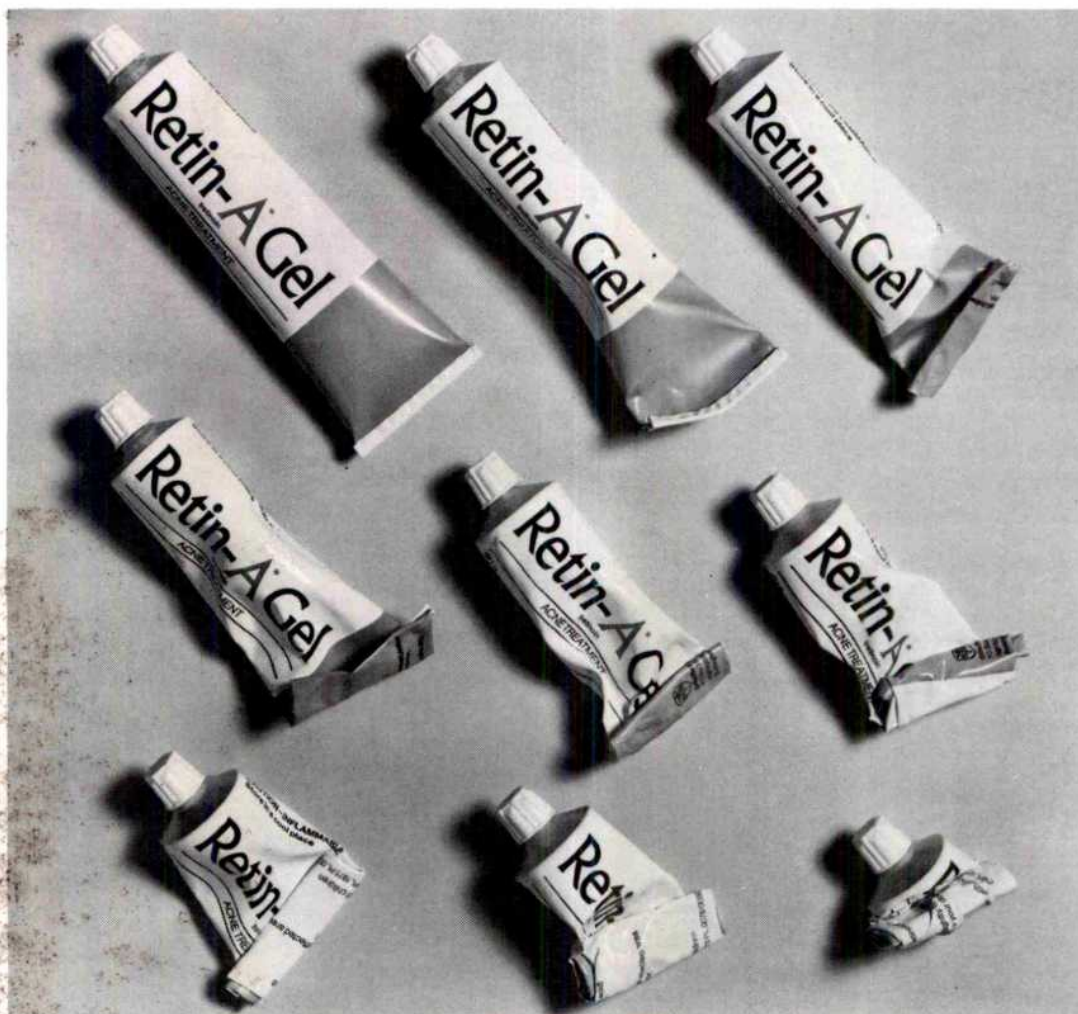
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1. Simpson, J. R. et al., (1973) Current Medical Research and Opinion, 1, 485. 2. Mills, O. H. et al., (1972) Archives of Dermatology, 106, 200. 3. Leyden, J. J. et al., (1974) Southern Medical Journal 67, 20. 4. Mandy, S. H. (1974) Cutis 14, 855-857.

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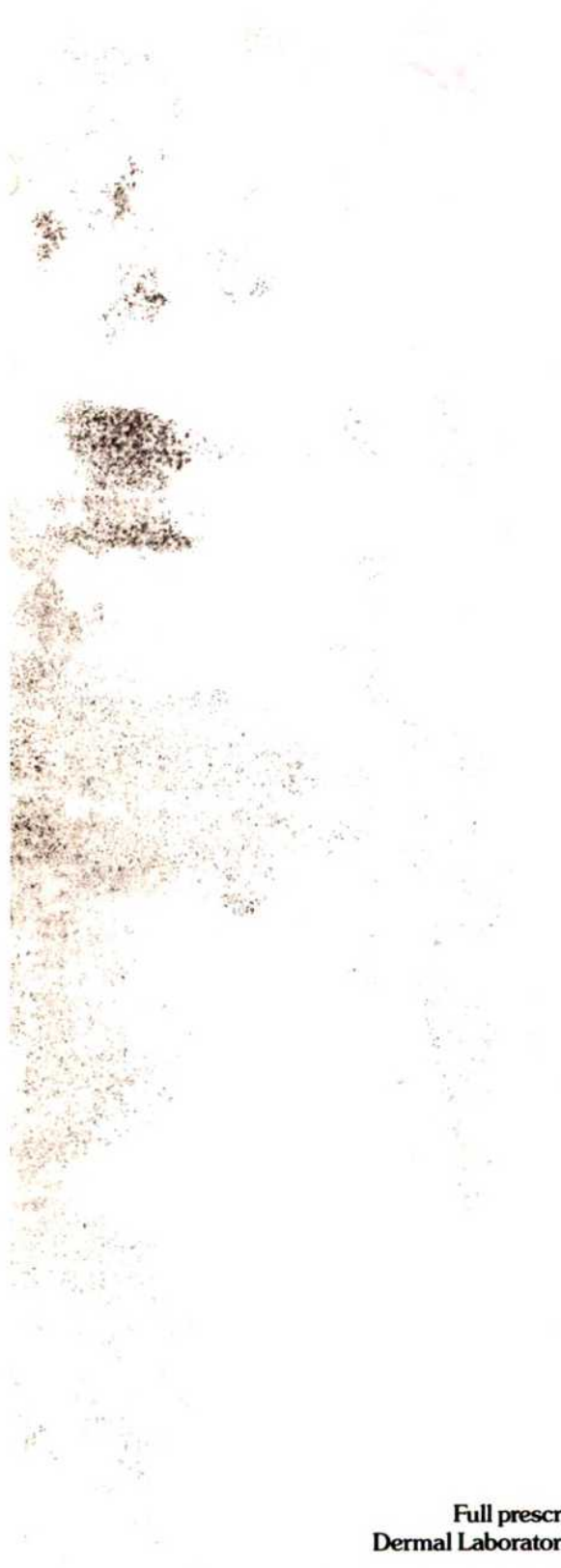


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Erythrokeratolysis hiemalis—Keratolytic winter erythema or 'Oudtshoorn Skin'

A NEW EPIDERMAL GENODERMATOSIS WITH ITS HISTOLOGICAL FEATURES

G.H.FINDLAY AND J.G.L.MORRISON

Section of Dermatology, University of Pretoria, Pretoria, South Africa

Accepted for publication 8 December 1977

SUMMARY

A new autosomal, dominantly inherited epidermal disorder is described in which a spreading dissection of the stratum corneum with redness of the palms and soles occurs as a result of a centrifugal necrobiosis of the Malpighian layer below it. It is seen characteristically in winter. In extensive cases the limbs and trunk are affected with gyrate scaling erythemas.

We are pursuing a preliminary investigation of a new dermatosis which has already been called erythrokeratolysis hiemalis or keratolytic winter erythema. Over a stretch of 20 years its distinctive clinical features have gradually come to light, and the histological characteristics are now reported for the first time from material gathered during the past 5 years. We possess some 8–10 apparently separate family trees with serological linkage studies of affected individuals. The likelihood of unifying them is increasing because they all converge in the early 19th Century upon the district of Oudtshoorn situated in what was then the Cape Colony. For local reasons we therefore call it 'Oudtshoorn Skin'. A general clinical account for a wider readership has appeared in a local journal with 6 colour illustrations (Findlay *et al.*, 1977).

INTRODUCTORY OBSERVATIONS

The hitherto unreported circumstances of our study are these:

First case. In 1957 we saw a 2-year-old boy with a pink exfoliative condition of the soles having loose, outlying, fungus-free blisters round the sides of the feet which vaguely suggested keratolysis exfoliativa or some type of epidermolysis. It developed during winter, and 3 other relatives—a grandparent, parent and aunt along the same family line—had a similar trouble on the palms and soles, also recurring in winter. This patient is today a healthy 22-year-old electronic technician who has found that his palms and soles are normal and dry in summer but at the onset of a cold winter they start

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sweating and begin to peel in red circinate patches. Nowadays the hands are worse affected than the feet. The horny layer appears to blister and loosen, and can be pulled off in strips without any pain, cracking, rawness or disability. Such an individual patch of centrifugal peeling remains active for about 6 weeks. More spots appear if the skin is softened by moisture such as occurs with sweating in closed shoes or gloves, or by immersion in water. Four generations back along the affected line the family lived near Oudtshoorn. Many affected relatives have been traced and examined other than those originally known to suffer from the disorder.

General background. For the period between 1955 and 1969 we hold many files on cases with palmo-plantar hyperhidrosis and keratolysis exfoliativa. Most of these belong to the better known summer complaint, possibly related to dyshidrotic eczema, with islands of tiny dry blisters and very fine centrifugal desquamation. Since 1969 we began to distinguish more clearly the new disorder with redness and larger peels. Going back on our records and those of our colleagues it is obvious that the disease which we are now considering, depending on its phase and site, had been grouped indiscriminately with such divergent conditions as erythema multiforme, Weber-Cockayne disease, congenital syphilis, Pernet's symmetrical lividity, dermatitis repens—not forgetting the humbler categories of psoriasis, eczema, pyoderma and ringworm.

Second family. In the winter of 1969 we saw a 16-month-old boy incapacitated by red, peeling, septic palms and soles, with a family history of the same trouble going back 4 if not 5 generations to a man of partly French extraction born in Oudtshoorn. This child, now 9-years-old, has taught us that the condition also presents with gyrate scaling lesions up the limbs, and that attacks can be triggered by sore throats. The diagnostic spectrum was thus expanded to include a disorderly assemblage of scaling and gyrate erythemas, *généodermatose en cocarde*, Hailey-Hailey disease, the dubious company of bacterides, etc. Several of this child's affected relatives have since been seen. One of his adult cousins had played rugby with our former assistant Dr Alan Menter, who marvelled at this man's ability to pull skins endlessly off his palms in winter before a rugby match. While in Pretoria Menter himself discovered another affected family with the same disorder.

Further findings. In each year since 1969, dermatologists in South Africa have unearthed more cases and families, many not known to be related as yet to our earlier families, although traceable to the same district. In Johannesburg Drs David Whiting and George Nurse constructed the largest family tree yet prepared, and traced the progenitors to Oudtshoorn. Quite independently of us Dr Edgar Swart discovered two affected families near Cape Town and recognized their uniqueness, while Dr Hymie Klevansky in Port Elizabeth had done the same. All cases came from the Oudtshoorn area. At present the extensive family histories are being brought together by Dr Peter Hull and the serology studied by Professor Trefor Jenkins.

Geography and genetics. In the early and mid-Victorian era, the Oudtshoorn district in the Cape Colony was mainly given to fruit, grain and tobacco farming, soon to expand with the establishment of a profitable ostrich industry. We must still discover how the disease we describe came to be introduced into this isolated community. It soon existed widely in the small, indigenous white farming population which was prolific and somewhat intermarried, and was explosively spread through their descendants whose daughters sometimes married young male immigrants from Britain and Europe. Many with 'Oudtshoorn Skin' moved away, and may by now be found anywhere in South Africa, while others are known from further afield in Rhodesia, Canada and the U.K.

The disorder is inherited as a monogenic autosomal dominant of high penetrance.



(a)



(b)



(c)



(d)

PLATE I. Erythrokeratolysis hiemalis.

(a) Appearance of the foot in boy aged 18 months. (b) Foot lesions in man aged 22. (c) Hand and thigh lesions in girl aged 10. (d) Buttock and thigh lesions in boy aged 8.

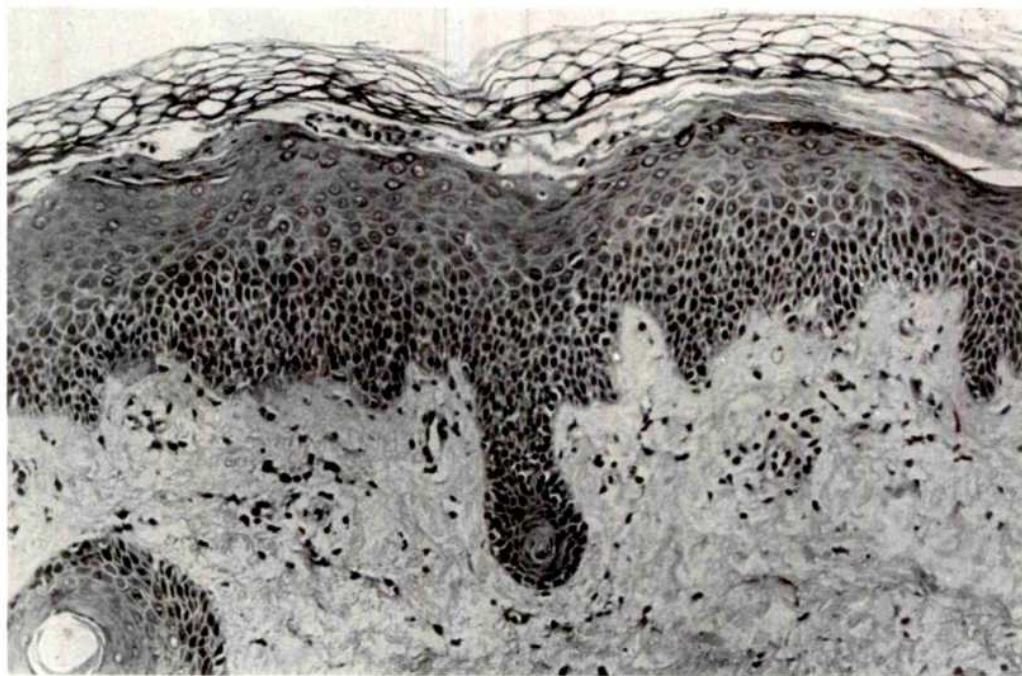


FIGURE 1. Erythrokeratolysis hiemalis—most of the representative epidermal changes can be seen here: (i) Basaloid proliferation ('basaliosis'); (ii) Reduction in the thickness (from left to right i.e. from periphery towards the centre) of the Malpighian layer; (iii) Granular layer absent and partly vacuolated in the younger (left half) part of the lesion and reconstituted in the older part to the right; (iv) Parakeratotic layer forming on the left, and compacted between the old and new stratum corneum on the right.

Supplementary clinical features. Extending the preliminary observations, we have found familial cases that are less seasonal, being either perennial or erratic in onset, and precipitated by fevers and surgical operations. The trouble often begins in infancy or during adolescence, becoming less bothersome in adult life until the signs are eventually rather trivial or entirely lacking in middle age. In our recent paper (Findlay *et al.*, 1977) we provide further clinical detail and some experiences with treatment.

Clinical summary of erythrokeratolysis hiemalis (Plate 1). It occurs in cyclical attacks, each lesion lasting about 2 months, of roughly symmetrical centrifugal erythematous macules which peel thickly from the centre outwards, liberating the full stratum corneum in the process. Before the horny layer separates the lesions look like dry vesicles or bullae, but may be exudative and infected on occasions. Palmo-plantar surfaces are the most constant sites, though spread round the hands and feet with affection of the dorsal and paronychia areas is common. When severe, the lesions appear on the limbs and sometimes on the trunk.

Histological material and methods

Sections stained routinely with haematoxylin and eosin from some 40 paraffin blocks of skin tissue were available from 18 patients with the disease. Two very early lesions were serially sectioned and 3

excisions showed the topography at the advancing edge of the lesion. Many of the patients were our own, but we are greatly indebted to our colleagues for supplying certain crucial sections and other corroborative slides.

Histological findings (Fig. 1)

In brief, the greatest damage occurs in the Malpighian layer, and the visible changes spread horizontally along it. Just at the outside of an advancing lesion there may be a little spongiosis and vesicle formation covered by an intact granular layer. In the rear of this region, there is a narrow zone where the most characteristic event takes place—necrobiosis of the Malpighian layer with absence of an overlying stratum granulosum. This zone becomes parakeratotic, and is ploughed out of the epidermis centrifugally by the recovering layers behind it. This damaged layer is hoisted up and becomes sandwiched between the old stratum corneum above and a new one formed below it. Elsewhere in the lesions the Malpighian layer often looks atrophic. The through-put of normal cells across it is evidently blocked, to judge by the accumulation of proliferated basal cells below these regions.

Considering the changes in more detail, the damaged Malpighian cells become pale-staining, shrunken and lytic, and reduced to 3–4 layers in thickness. Nuclear changes are most obvious and comprise lysis or pyknosis with fragmentation. Necrosis of whole cells is seen here and there, occasionally with colloid transformation of a cell group or release of some eosinophilic granular debris. This necrobiotic change takes place in two directions. The cells either become compacted together and tend to lie parallel to the surface as if about to become parakeratotic, or they remain looser, with a little spongiosis which along with the cellular necrobiosis may result in a primordial eczema vesicle.

As the Malpighian layer is damaged centrifugally, the epidermis seals up the damage, pushing out a continuous compact parakeratotic lamina from behind. The resulting lamina consists of 2–8 cell layers. Along this plane the horny layer splits off, producing the characteristic thick peeling. Parakeratosis therefore arises from the necrobiotic Malpighian layer ejected through a migrating gap in the stratum granulosum. As this abnormal layer is lifted out, it may carry the occasional eczema vesicle enclosed intact within its strata. These parakeratotic cells are mostly filled with a large quantity of minute round particles or droplets which are silvery under phase contrast microscopy. Polymorphs and cocci are apt to appear on either side of the parakeratotic sandwich, resulting in various secondary changes.

A sharp proliferation of epidermal basal cells commences next to the area of parakeratosis in the Malpighian layer. This basaloid proliferation occurs with a persistent atrophy of the Malpighian layer above it, much as if a regenerative stimulus to the basal cells was coupled with a block in their maturation. Towards the centre of a gyrate lesion the normal balance between the epidermal layers is gradually restored.

Basaloid proliferation, or 'basaliosis' as we may call it, comprises an accumulation of basal cells 6–8 layers thick. Acanthosis and prolongation of the rete ridges are significantly absent. Instead, the proliferating basal cells form vertically arranged block-like masses which efface the papillae and engulf the melanocytes. Mitoses are frequent and can be seen several layers above the basement membrane. These proliferating basal cells are not always normal: they may be spongiotic and necrobiotic with a tendency to form a further wave of microscopic eczema-like vesicles. Melanocytes with abundant dendrites are active. Pigment granules move up in all layers to the surface with none going into the dermis. The basement membrane region may be oedematous and vacuolated.

The granular layer thickens in the wake of the parakeratotically transformed Malpighian layer. A necrobiosis of granular cells is sometimes observed on the external surface of this increased layer. These cells have the shape and size of granular cells, but lose their granules and nucleus, leaving a pale, non-cornified cytoplasmic mesh. This monolayer of granular cell ghosts occupies the position of a stratum lucidum.

The old and new horny layers are not visibly abnormal despite occasional infection and polymorph accumulation.

The dermis is minimally affected, and shows capillary congestion, pericapillary oedema and a trivial infiltrate lying behind the advancing epidermal lesion.

COMMENT

The newly recognized condition which we describe here is perhaps best considered as a disorder of skin shedding or as a process of intra-epidermal rejection. Most mammals shed their skin and hair in a piecemeal fashion. Moulting and sloughing require the intervention of a widespread 'growth dissociation.' In erythrokeratolysis hiemalis the phenomenon apparently takes place by a necrobiosis propagated in the Malpighian layer which is ejected through a shifting hole in the stratum granulosum. The granular layer following in its wake is in several areas flanked by Malpighian atrophy inside and granular cell 'ghosts' on the outside. It makes one ask if the formation of the granular layer is not induced by stimuli diffusing inward as well as the more usual idea that it ripens from within outward.

Factors in the initiation and control of moulting in reptiles are temperature, moisture, nutrition and endocrine (thyroid) changes (Bellairs, 1969). In man erythrokeratolysis hiemalis harks back to a disorder of shedding, to which bacterial infection can, in susceptible individuals, be added to the list of triggering factors.

The nearest approach to the disease we describe, despite many differences in detail, is provided by the g nodermatose en cocarde (Degos *et al.*, 1947) with its large, rounded erythematous and scaly plaques, particularly of the legs. One of their cases, we are incidentally told, showed that 'la paume des mains est le si ge d'une desquamation l g re de type desquamation estivale en aires; il n'y a pas de keratodermie'. From Gougerot & Grupper (1948) we learn that '... pendant la saison chaude les l sions disparaissent compl tement pour r appara tre en automne et conna tre leur maximum d'extension pendant la saison froide'. Describing 2 cases of erythema perstans (similar perhaps to the erythema gyratum perstans of Colcott Fox) Wende (1906) in America noted a distinct aggravation of the trouble in cold weather. Whatever the standing of these antiquarian clinical entities, it is at least intriguing to ask if Degos' disease among newer descriptions could not be the same as ours. Suppose a French immigrant introduced the causative gene long ago into the Oudtshoorn community, the clinical picture could shift among the descendants towards the patterns we now see. A common ancestor for the scores of present-day sufferers has not yet been identified but there is already some promise, and even likelihood, that it was a Frenchman.

ACKNOWLEDGMENTS

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REFERENCES

- BELLAIRS, A. (1969) Skin replacement. In: *The Life of Reptiles*, vol. 2, pp. 289-293. Weidenfeld and Nicolson, London.
- DEGOS, R., DELZANT, O. & MORIVAL, H. (1947) Eryth me desquamatif en plaques, cong nital et familial (g nodermatose nouvelle?) *Bulletin de la Soci t  fran aise de Dermatologie et de Syphiligraphie*, **54**, 442.
- FINDLAY, G.H., NURSE, G.T. *et al.* (1977) Keratolytic winter erythema or Oudtshoorn disease. *South African Medical Journal*, **52**, 871.
- GOUGEROT, H. & GRUPPER, Ch. (1948) G nodermatose  ryth matosquameuse circin e, variable: 'Maladie de Degos'. *Bulletin de la Soci t  fran aise de Dermatologie et de Syphiligraphie*, **55**, 396.
- WENDE, G.W. (1906) Erythema perstans, with a report of 2 cases involving circinate lesions. *Journal of Cutaneous Diseases*, **24**, 241.

Chilblain lupus erythematosus (Hutchinson)

A CLINICAL AND LABORATORY STUDY OF 17 PATIENTS

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SUMMARY

Chilblain lupus erythematosus (LE) is a chronic unremitting form of LE seen predominantly in women. It occurs commonly on the digits, calves and heels. Nasal lesions are rare. Chronic facial discoid LE usually appears before the chilblain form but, in most instances, resolves even though the chilblain lesions persist. Transformation to systemic LE occurs more often in those who develop both forms of cutaneous LE simultaneously and in the erythema multiforme syndrome. The chilblain lesions are the result of microvascular injury secondary to exposure to cold and possibly hyperviscosity from immunological abnormalities. Elevated serum gammaglobulins, positive latex factor and speckled pattern antinuclear factor are common. None of the usual treatments for cutaneous LE is effective for the chilblain lesions, probably because none is directed towards prevention and treatment of the microvascular stasis.

In 1888 Jonathan Hutchinson described chilblain, or perniosis, lupus erythematosus (LE) in his classification of LE (Fig. 1). He described 'the proclivity to chilblains. The tendency on the part of the tissues to inflame and become irritable, under the intermittent influence of cold and damp, which is so marked in many cases of lupus that this disease might be regarded as being a modified chilblain. This statement concerns the erythematous form more frequently than it does the common lupus'.

There are few figures of incidence available. Gold (1960) found 3% in a large series of chronic discoid LE. These cases are mentioned in passing and there has been little or no discussion on the natural history or prognosis of this form of LE. Even the major texts (Dubois, 1976) give no more than a brief mention to the condition.

This paper describes the clinical and laboratory features of 17 cases of perniosis or chilblain LE, seen in a review of 150 cases of cutaneous LE presenting in the last 20 years. This group of 17 patients includes the 4 patients who developed erythema multiforme lesions (Rowell, Beck & Anderson, 1963) and these will be considered as a group.

Clinical features

The important features are summarized in Table 1. All 17 patients were female. There are 3



FIGURE 1. Chilblain lupus erythematosus showing lesions on dorsum of hand (from Hutchinson's *Clinical Illustrations*, *Archives of Surgery*, Vol. IV, 1893).

discernible groups. Eleven out of 17 presented with chilblain LE after the development of facial discoid LE and the delay varied from 1 to 14 years (mean 5.9 years). Four presented with both forms of LE simultaneously and 2 with perniosis LE alone. The 4 patients who showed the LE erythema multiforme syndrome are indicated. The particular features of this condition were described by Rowell *et al.* in 1963. Age at onset of the whole series was from 20 to 56 years although only 5 patients were over 30 years at onset. Three patients had chilblains and bad circulation before the onset of LE. The sites of the chilblain lesion have mainly been on the fingers and toes as expected (Table 2). Typical lesions in the fingers are shown in Fig. 2. They consist of a purplish blue discolouration over the knuckles, especially over the terminal I.P. joints on the dorsa of the phalanges, with little hyperkeratosis. Fissuring occurs commonly over the knuckles and dorsa of the toes (Fig. 2). The lesions on the pulps of the fingers are atrophic and macular (Fig. 3), do not ulcerate like vasculitic lesions and very rarely fissure. The toes show these features but in some cases a purpuric element occurs (Fig. 4). The fifth finger and fifth toe are most frequently and severely involved and may be the only digits affected, as in 2 cases in the series.

Fissuring of the heels causes considerable discomfort. This may occur without the toes on the foot being affected (Cases 2, 10, 11). Lesions on the calves, knees and elbows are seen less often. Chilblain LE on the nose is rare and was present in only one patient.

TABLE 1. Clinical features of chilblain LE

Case no.	Age of onset (years)	Presenting form of LE	Site of original LE	Onset of chilblain LE (years)	Site of chilblains	Duration of original LE (years)	Duration of chilblains (years)	SLE	Other features
1	27	Discoid	Cheeks, nose	14	(R) toes, (L) knee	20	7	No	
2	29	Discoid	Scalp, forehead, cheeks	10	Heels	16	7	No	
3	23	Discoid	Forehead, nose, cheeks	2	Toes, heels, (R) fingers	15	13	Yes	
4	22	Discoid	Cheeks, forehead	6	(L) elbow	9	3	No	
5	22	Discoid	Cheeks, chin	9	Fingers, toes	19	10	No	
6	24	Discoid	Cheeks, ears	5	Fingers, toes, heels	3	7	No	
7	46	Discoid, rosaceous	Face	4	Calves, toes	15	10	No	Died. Monocytic leukaemia
8	26	Discoid	Cheeks	1	(R) calf	9	8	No	Erythema multiforme
9	56	Discoid	Cheeks	4	Hands, forearm, feet, heels	22	27	No	Erythema multiforme
10	28	Discoid	Cheeks, nose	3	Calves, heels	4	5	No	Erythema multiforme
11	36	Discoid	Scalp, nose	7	Nose, heels	14	21	No	
12	20	Discoid, chilblain	Cheeks	S	Calves, neck, (R) small finger	9	15	No	Nail dystrophy
13	28	Discoid, chilblain	Arms, legs	S	Toes, heels, calves	27	27	Yes	Erythema multiforme
14	15	Discoid, chilblain	Cheeks, nose, ears	S	Hands, calves	27	27	Yes	
15	33	Discoid, chilblain	Cheeks	S	Fingers	1	2	No	
16	52	Chilblain	—	—	Fingers	—	36	No	Marked nail dystrophy
17	21	Chilblain	—	—	Feet	—	6	No	

S = simultaneously.

Progress of pernioic lesions in relation to discoid LE elsewhere

In 8 of the 11 patients who developed chilblains after facial discoid lesions, discoid LE resolved after 3–22 years (mean 13.3 years). The chilblain lesions have continued without resolution in all 11 patients. Of the 4 patients in whom both types of LE presented simultaneously, 2 progressed to SLE without regression of either skin lesion. The chilblain lesions persisted in the other two although the discoid lesions remitted in one after eight years. Those 2 patients with pernioic LE alone showed no resolu-

TABLE 2. Site of chilblain LE

	No. of patients
Toes	8
Fingers	8
Heels	8
Calves	5
Elbows	1
Knees	1
Nose	1



FIGURE 2. Chilblain lupus erythematosus on dorsum of hand with typical fissuring over the joints.



FIGURE 3. Atrophic lesions on pulps of fingers.

tion. One patient (Case 16) seen 36 years after the onset of eruptions showed active disease and gross distortion of the finger tips and nail beds (Fig. 5).

Perniotic LE in those patients with the erythema multiforme syndrome

The erythema multiforme syndrome (Rowell *et al.*, 1963) was originally described in patients with discoid LE who have associated chilblain lesions and characteristic immunological abnormalities. These are the presence in the serum of 'speckled' type of antinuclear factor, a precipitating antibody to saline extract of human tissues, and rheumatoid factor. Subsequent cases have been reported by Jablonska, Blaszczyk & Chorzelski (1972) and by Fabbri & Panconesi (1975). The original patients have been followed up for 5–27 years. All 4 continued to have troublesome lesions, particularly in the winter, but sometimes in the summer. One patient (Case 13) developed systemic LE, which was controlled by prednisone, eight years after the onset of cutaneous LE. She died 19 years later from a coronary thrombosis. Another patient (Case 8) was followed up for 8 years and, although she did not develop SLE, positive speckled antinuclear factor persisted. There was no arthralgia and the erythema multiforme lesions remitted without systemic steroids, although her chilblain LE remained



FIGURE 4. Chilblain lesions showing purpuric element on sole of foot.



FIGURE 5. Gross nail dystrophy after 36 years' activity.

unchanged. Speckled antinuclear antibody was identified at birth in her second child and persisted for 1 year, during which marked photosensitivity of face and hands were noted. The third patient (Case 10) showed no signs of systemic involvement although she had a permanently positive speckled antinuclear factor of 1:1000. She suffered a transient left hemiparesis at the age of 34 but there was no evidence of SLE at the time. She was lost to follow up 2 years later. The fourth and oldest patient in this group is now 83-years-old. The discoid lesions remitted after 22 years but she still suffers from chilblains on her feet and calves. If she stops taking 5 mg prednisone daily the erythema multiforme lesions return. She no longer has a positive speckled antinuclear factor although her latex test is still positive.

Transformation to systemic LE

Three of the 17 patients (17.6%) developed SLE according to the criteria of the American Rheumatism Association (Cohen & Canoso, 1972). In all 3 patients the onset of the pernitiotic and discoid LE was close. In two, both patterns appeared simultaneously and, in the other, only two years apart. The development of systemic LE was slow and occurred at 5, 8 and 18 years. All three suffered arthralgia with positive latex tests. Two had frank manic depressive psychoses whilst the third showed periods of emotional instability. In all cases speckled antinuclear factor was demonstrated. The latter was part of the LE erythema multiforme syndrome in Case 13. Case 14 now has a reduced creatinine clearance (60 ml/min) presumably from renal involvement.

Laboratory investigations (Table 3)

Six of the 17 patients had a low haemoglobin on presentation and 4 of these had associated erythema multiforme. In none, however, was there a leukopenia or thrombocytopenia. The ESR was raised (> 20 mm/h) in six patients. This was related to anaemia in two, Cases 9 and 13. The latter patient developed SLE.

Only five patients showed normal levels of serum gammaglobulins. Quantitative serum immunoglobulins in eight patients showed a variety of changes (Table 3). It is notable that both patients with a combined elevation of IgG and IgM developed SLE. Although a positive latex test was demonstrated in 9 out of 17 cases this was not necessarily in those with raised gammaglobulins. This group included all three with elevated IgG, all four with the erythema multiforme and two others who developed SLE. The latter 6 patients also had permanently positive speckled antinuclear factor in high titre. Joint symptoms were present in only three patients who developed SLE, and in Case 9. The other five patients had no arthralgia or arthritis despite positive latex tests for, in some cases, over 10 years.

Cryoglobulins were not detected in any patient. This investigation was repeated many times during active and quiescent phases of the disease. Cold agglutinins were found in Case 7 but bore no relationship to the severity of her disease. She eventually died of monocytic leukaemia. Case 1 has had a persistently positive WR for 20 years in association with discoid LE but has not developed the systemic form.

Histology

Histological sections were available from 8 patients. The epidermis was atrophic with some follicular plugging. There was little hyperkeratosis or acanthosis which was consistent with the smooth atrophic clinical appearance, although biopsies from the heel showed hyperkeratosis. The stratum granulosum was barely evident and there was extensive liquefaction of the basal epidermis. Lymphocytes predominated in the dermal infiltrate and were seen around small blood vessels. Direct immunofluorescence of involved skin (Dr D.G.Scott) was performed on 3 patients. Granular linear staining for IgG and IgM was seen at the dermo-epidermal junction in all of them. In addition one patient (Case 14) showed positive staining for C3. Globular aggregates of IgG and IgM were seen around dermal blood vessels in Case 5 but these were sparse and unlike the cytoid bodies found in lichen planus. There was no staining for fibrin.

TABLE 3. Laboratory investigations in chilblain LE

Case no.	FBC	ESR mm/h	Cryoglobulins	Cold agglutinins	WR	Gammaglobulin	Latex	Antinuclear factor
1	N	36	—	—	+	N	—	—
2	Hb 77%	2	—	—	—	N	—	—
3	N	16	—	—	—	↑IgG↑IgM	+	Speckled 1:1000
4	N	5	—	—	—	↑IgA	—	—
5	N	15	—	—	—	↑IgM	—	—
6	N	19	—	—	—	↑IgG↑IgM↑IgA	+	—
7	Hb 70%	15	—	+	—	Increased	+	—
8	Hb 70%	18	—	—	—	Increased	+	Speckled 1:256
9	Hb 76%	40	—	—	—	↑IgM	+	Speckled 1:16
10	N	26	—	—	—	Increased	+	Speckled 1:1000
11	N	37	—	—	—	↑IgA↑IgM	—	—
12	Hb 84%	10	—	—	—	N	+	—
13	Hb 66%	49	—	—	—	Increased	+	Speckled 1:256
14	N	6	—	—	—	↑IgG↑IgM	+	Speckled 1:100
15	N	30	—	—	—	N	—	—
16	N	4	—	—	—	↓IgA	—	—
17	N	8	—	—	—	N	—	—

N = Normal.
Hb = Haemoglobin.

Effect of therapy

This was universally disappointing. Whilst topical steroids were effective for the discoid lesions, only 2 patients claimed a consistent significant effect on the chilblain lesions. Topical steroids under polythene were of no additional benefit. Intralesional triamcinolone was not used. Two out of 5 patients were helped by systemic steroids although one needed high doses (prednisone 25 mg daily). The two patients with the erythema multiforme syndrome who took prednisone showed complete suppression of the ringed lesions with small (5–10 mg) daily doses, but this had no effect on the perniois.

The antimalarials, chloroquine and hydroxychloroquine, were of benefit to only 3 patients. Both the discoid and the chilblain form responded but relapsed immediately the therapy was stopped. Two patients (Cases 6 and 7) did not benefit from a further course. Gold was responsible for the improvement in Case 10 but, unfortunately, she developed a sensitivity eruption and the treatment could not be repeated. Vasodilator drugs, e.g. nicotinic acid, naftidrofuryl and thymoxamine were of no benefit. All patients agreed that the best therapy was a hot summer. Case 12 took whisky four times a day when the lesions were bad and found this the best treatment of all.

DISCUSSION

These 17 patients represent 11.3% of a series of 150 patients with cutaneous LE seen during a follow-up survey of patients originally attending the Dermatology Clinic during the years 1958–62. The incidence of chilblain lesions is surprisingly high considering the scant attention it has merited in the past. This is probably because the chilblain lesions are the presenting features in only a third of patients. The condition may be missed unless specifically looked for, and many patients regard chil-

blains as a troublesome, though trivial, phenomenon. The climate may not be cold enough to produce the lesions and this accounts for the fact that Tuffanelli & Dubois (1964) in California saw no lesions of this type in a large series of 520 patients.

The majority of lesions appear below the knee, especially on the toes, heels and calves. Most clinical descriptions emphasize the peripheral involvement but this series would not support the usual view that the nose and external ear are commonly involved. The extent and number of lesions are independent of disease severity. The three patients who developed SLE had pernicious lesions indistinguishable from the remainder of the group.

The presence of Raynaud's phenomenon is of no significance either. The common feature was the persistence of this form of LE. Once the chilblain appeared the patients continued to suffer even though in the first group (Cases 1-11) the discoid LE remitted. Where the two forms of the disease appeared simultaneously two of the four patients developed SLE later. The third patient who developed SLE had discoid lesions followed two years later by chilblains, compared with the mean delay for the group of about 6 years.

Why are all the patients female? Possibly this reflects the fact that women are more vulnerable to chilblains because of the distribution of their normal body fat. However, there is a female preponderance in LE, particularly in the systemic form, where the female: male ratio may approach 10:1. The precise contribution is, however, difficult to assess. Transformation to SLE occurred in three (17.6%). The normal rate for patients with discoid LE in this area is less than 5% (Rowell, 1972) but the number of patients is too small to indicate a definite increased risk.

The particular form of the SLE is interesting. Two developed manic depressive psychoses whilst the third was treated for intermittent endogenous depression for the last 5 years of her life. Renal involvement, which was mild, occurred in one case only. Antinuclear factor was of the speckled pattern. This is unusual for SLE where the homogeneous type usually predominates (Rowell, 1972; Jablonska & Chorzelski, 1974). This speckled antinuclear factor was also found as part of the erythema multiforme syndrome (Rowell *et al.*, 1963) and as such may be part of the immunological disturbances which contribute to the formation of the chilblain lesions. One of the patients with erythema multiforme developed SLE. This association has been noted previously (Jablonska *et al.*, 1972). The pattern of immunoglobulin response was interesting. The 2 patients with SLE showed the typical pattern of raised IgG and IgM (Alarcon Segovia & Fishbein, 1972). The remainder of these patients had an IgM and/or IgA response. Fraser, Dick & Chrichton (1969) demonstrated that serum IgA was significantly raised in active discoid LE although there were no data on chilblain lesions.

The presence of positive rheumatoid factor in over half the patients was significantly high compared with a previous study of discoid LE (Beck & Rowell, 1966). None of these patients developed arthritis although 4 had persistent arthralgia. Cryoglobulins were absent in all cases. This is not usual in discoid LE (Gentele, Lagerholm & Lodin, 1959). Cold agglutinins were found in Case 7 and these were persistently positive until she died from monocytic leukaemia.

The association of chilblain lesions and a monocytosis in the blood seen in Case 13 has been recorded by Marks, Linn & Borrie (1969). They described three patients with chilblains, leukopenia, and a monocytosis which were possibly manifestations of a preleukaemic state. In none of these three were leukaemic cells detected in the peripheral blood. One of these three had definite chilblain LE and our patient probably represents the end stage of the disease suspected but not seen by these authors. The preleukaemic stage may show as minor abnormalities in the peripheral blood for some time before developing into a frank leukaemia. In this case the delay was 5 years.

Lesions appearing after a fall in temperature may be produced by immunological abnormalities even though these are not especially temperature dependent. Increased concentration of gammaglobulin and the presence of rheumatoid factor increase the viscosity of the serum (Shearn, Epstein & Engleman, 1963). These two proteins appear to exert an independent effect upon viscosity. Rheumatoid factor affects viscosity in spite of its small concentration because of its large molecular weight. Superimposed on this, cooling of the blood within the capillary loop will increase blood viscosity and slow

blood flow further. The changes that occur in blood viscosity affect the microvascular pattern of the skin and determine the nature of the pathology which results from stasis (Ryan & Copeman, 1969). Chilblains are found in areas protected from the body heat by fat pads or on anatomically isolated sites such as the digits.

Platelet adhesiveness and aggregation are increased by cold and retard the free flow of blood through narrow channels. Although there was no direct evidence of this phenomenon occurring in these patients, Shrank (1964) described a case of chilblain LE where the attacks of chilblains coincided with a fall in platelet count and reduced platelet half life.

It is not surprising that topical steroids produce little benefit in chilblain LE. They suppress the cutaneous inflammation after the damage has been done. Systemic steroids work in some patients presumably by reducing the immunological abnormalities, but the dangers are obvious. Among other actions antimalarials have a desludging effect on aggregated red cells (Cecci & Ferraris, 1962) and it may be this which has helped some. However, treatment directed towards reducing blood viscosity and preventing microvascular injury may be more rewarding.

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REFERENCES

- ALARCON SEGOVIA, D. & FISHBEIN, E. (1972) Serum immunoglobulins in systemic lupus erythematosus. *Clinical Science*, **43**, 121.
- BECK, J.S. & ROWELL, N.R. (1966) Discoid lupus erythematosus. *Quarterly Journal of Medicine*, **35**, 119.
- CECCI, E. & FERRARIS, E. (1962) Desludging action of hydroxychloroquine in rheumatoid arthritis. *Acta Rheumatica Scandinavica*, **8**, 214.
- COHEN, A.S. & CANOSO, J.J. (1972) Criteria for the classification of systemic lupus erythematosus. *Arthritis and Rheumatism*, **15**, 540.
- DUBOIS, E.L. (1976) *Lupus Erythematosus*, 2nd edn. (revised), p. 215. University of Southern California Press.
- FABRI, P. & PANCONESI, E. (1975) Syndrome de Rowell. Étude clinique et immunopathologique de deux cas. *Annales de Dermatologie et de Syphiligraphie*, **102**, 405.
- FRASER, N.G., DICK, H.M. & CRICHTON, W.B. (1969) Immunoglobulins in dermatitis herpetiformis and various other skin diseases. *British Journal of Dermatology*, **81**, 89.
- GENTILE, H., LAGERHOLM, B. & LODIN, A. (1959) Cryoglobulins in chronic discoid lupus erythematosus. *Acta dermato-venereologica*, **39**, 207.
- GOLD, S. (1960) Progress in the understanding of lupus erythematosus. *British Journal of Dermatology*, **72**, 231.
- HUTCHINSON, J. (1888) Harvean lectures on lupus. *British Medical Journal*, **1**, 10, 58, 111.
- JABLONSKA, S., BLASZCZYK, M. & CHORZELSKI, T. (1972) Syndrome de Rowell-Lupus érythémateux avec des lésions coexistantes de type érythème polymorphe bulleux. *Médecine et Hygiène*, **30**, 1390.
- JABLONSKA, S. & CHORZELSKI, T.P. (1974) Lupus erythematosus. In: *Immunological Aspects of Skin Diseases* (Ed. by L. Fry and P. Seah), p. 75. Medical and Technical Publishing Co., Lancaster.
- MARKS, R., LINN, C.C. & BORRIE, P.F. (1969) A pernicious syndrome with monocytosis and leukopaenia. A possible association with a preleukaemic state. *British Journal of Dermatology*, **81**, 327.
- ROWELL, N.R. (1972) Lupus erythematosus, scleroderma and dermatomyositis. In: *Textbook of Dermatology* (Ed. by A. Rook, D.S. Wilkinson and F.J.G. Ebling), vol. 2, p. 1061. Blackwell Scientific Publications, Oxford.
- ROWELL, N.R., BECK, J.S. & ANDERSON, J.R. (1963) Lupus erythematosus and erythema multiforme-like lesions. *Archives of Dermatology*, **88**, 176.
- RYAN, T.J. & COPEMAN, P.W.M. (1969) Microvascular pattern and blood stasis in skin disease. *British Journal of Dermatology*, **81**, 563.
- SHEARN, M.A., EPSTEIN, W.V. & ENGLEMAN, E.P. (1963) Relationship of serum proteins and rheumatoid factor to serum viscosity in rheumatic diseases. *Journal of Laboratory and Clinical Medicine*, **61**, 677.
- SHRANK, A.B. (1964) Chilblain lupus erythematosus. *Proceedings of the Royal Society of Medicine*, **57**, 515.
- TUFFANELLI, D.L. & DUBOIS, E.L. (1964) Cutaneous manifestations of systemic lupus erythematosus. *Archives of Dermatology*, **90**, 377.

Verrucous lesions in patients with discoid lupus erythematosus

CLINICAL, HISTOPATHOLOGICAL AND
IMMUNOFLUORESCENCE STUDIES

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SUMMARY

Seven cases of classical discoid lupus erythematosus of the face associated with hyperkeratotic, papulo-nodular lesions on the arms and hands were studied. Clinically the hyperkeratotic lesions resembled keratoacanthomas or hypertrophic lichen planus. The clinical course was marked by chronicity, an absence of regression of the lesions, and resistance to treatment. Histopathologically, a lichenoid cellular reaction seemed to play a key role in the development of the hypertrophic lesions which resembled either keratoacanthomas or hypertrophic lichen planus. Elastic fibres were prominent in the upper dermis, the lower levels of the epidermis and in the hyperkeratotic horny layer. Immunofluorescence of the papulo-nodular lesions demonstrated the deposition of IgG and IgM in a globular pattern at the dermal-epidermal junction. Discontinuous deposition of these antibodies was also seen at the basement membrane zone. On the basis of the immunofluorescence, histopathological and clinical studies, we suggest that the verrucous, hyperkeratotic lesions on the upper extremities represent an unusual, but distinct, form of discoid lupus erythematosus.

Discoid lupus erythematosus (LE) includes a broad spectrum of clinically distinct types of lesions (Tuffanelli, 1972). In addition to the more classical variety of discoid LE characterized by atrophy, telangiectasia, scaling and follicular plugging, both hypertrophic and verrucous lesions may occur as part of the spectrum of this disease. Bechet described a variant of discoid LE characterized by hyperkeratotic, erythematous plaques with predilection sites on the face (Bechet, 1940, 1942, 1950), which he called lupus erythematosus hypertrophicus et profundus. Even earlier, Kaposi recognized a form of lupus erythematosus mainly affecting the dorsal aspects of the legs (Kaposi, 1883). This entity was further delineated by Irgang (1940), and is now known as lupus profundus of Kaposi.

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Irgang (Otani, 1977; Feverman & Halevy, 1977). In addition to these recognized entities, there have been several case reports describing hypertrophic or atrophic lesions which have the clinical and histological features of both lupus erythematosus and lichen planus (Copeman *et al.*, 1970; Thormann, 1974; Umbert *et al.*, 1974; Davies *et al.*, 1977; Romero *et al.*, 1977). However, the relationship between the lichen planus-like lesions and discoid lupus erythematosus has not been clarified.

In this study we have examined seven patients with typical lesions of discoid LE involving the face and scalp, associated with multiple verrucous papulo-nodular lesions principally located on the upper extremities. On the basis of the clinical, histopathological and immunofluorescence observations, we suggest that these lesions represent verrucous variants of discoid lupus erythematosus.

MATERIALS AND METHODS

The seven patients included in the present study were seen at the Dermatology out-patient clinic of Barnes Hospital, Washington University Medical Center, St Louis, Missouri. The clinical lesions were carefully described and photographed at various stages of the study. Extensive laboratory studies were performed to evaluate the disease and pertinent findings are presented in the respective case reports. Biopsy specimens of skin for histopathological and immunofluorescent studies were obtained under local anaesthesia with 1% lidocaine. Skin biopsies for conventional light microscopy were fixed in 10% formalin and processed routinely. The sections were stained with Haematoxylin-Eosin, PAS (without and with digestion with diastase for 1 h at 37°C), Verhoeff-van Gieson, Orcein-Giemsa and Iron-Gallein. Specimens for immunofluorescence studies were immediately frozen in liquid nitrogen and after sectioning tissue specimens were stained by the direct immunofluorescent technique. The sections were rinsed in phosphate buffered saline (PBS) for 15 min after which they were incubated with fluorescein-labelled antibody, filtered on a Millipore filter, in a moist chamber for 20 min. The sections were rinsed with PBS for 30 min. and then mounted in glycerol and PBS (1:9). The anti-immunoglobulin-fluorescein conjugates, including goat anti-human IgG, IgM, IgA, complement (BIC/BIA) and fibrinogen, were obtained commercially.* The original specific antibody concentrations were 0.9 mg/ml, 2.5 mg/ml, 1.5 mg/ml, 0.63 mg/ml and 1.5 mg/ml, respectively, and they were diluted 1:8 with PBS. The fluorescein/protein ratios in the same preparations were 2.9, 1.8, 3.5, 2.5 and 2.8. The sections were examined under a Leitz Orthoplan fluorescence microscope.

CASE REPORTS

A summary of the clinical data is presented in Table 1; more detailed case reports are as follows.

Case 1. A 49-year-old male presented with a history of facial lesions since 1951 which were diagnosed initially as discoid LE. Within 2 years after the appearance of the facial lesions, the patient also developed numerous verrucous papules on his arms which were interpreted from a skin biopsy in 1974 as hypertrophic lichen planus. The patient was hospitalized in Barnes Hospital in July 1976 for an ulcer of the right leg. Physical examination demonstrated facial lesions consisting of areas of atrophy with surrounding erythema, telangiectasia, scaling and follicular plugging (Fig. 1). On the dorsa of both hands were numerous papules 0.5–1.5 cm in size. The lesions were hyperkeratotic, erythematous, and some demonstrated verrucous, keratinous plugs in the centre. They were also non-pruritic. The papules extended up to the elbows on both forearms where they tended to coalesce into large hyperkeratotic

* Antibodies to IgG and complement were obtained from Meloy Laboratories, Inc., Springfield, Virginia. Antibodies to IgM, IgA and fibrinogen were obtained from Hyland Division, Travenol Laboratories, Inc., Costa Mesa, Cal.

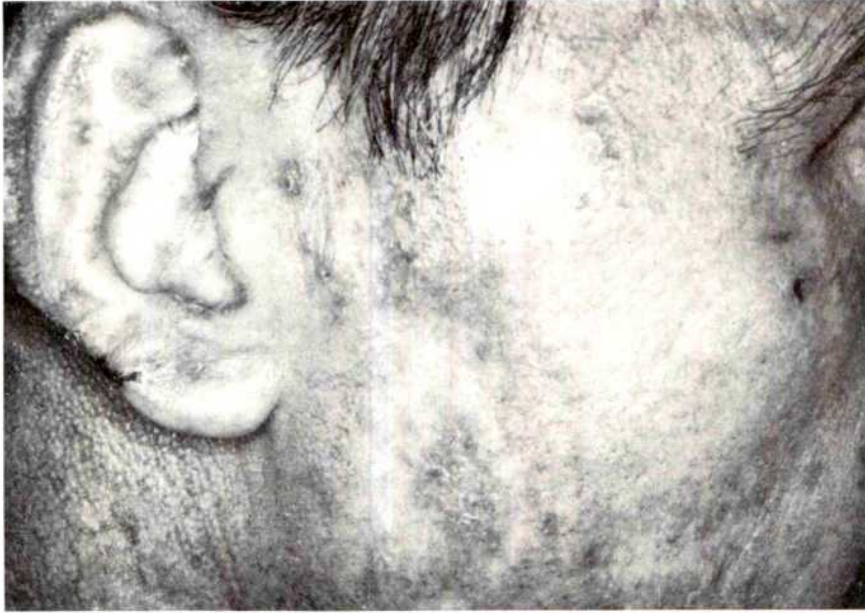


FIGURE 1. Lesions on the face typical of discoid lupus erythematosus, showing scarring, atrophy, follicular plugging and telangiectasia (Case 1).



FIGURE 2. Lesions on the arms and chest with numerous hyperkeratotic papules. The atrophic areas on the left arm are secondary to treatment (Case 2).



FIGURE 3. A detail of the left hand of the patient shown in Fig. 2.

TABLE 1. Clinical data of patients with atrophic and verrucous lesions

Case no.	Age (years)	Sex	Duration of disease (years)	Site of atrophic lesions	Site of verrucous lesions	ANA	Effective treatment
1	49	M	15	Face	Arms, dorsa of hands	—	Intralesional triamcinolone acetonide
2	45	M	15	Face, chest, arms	Arms, dorsa of hands	—	—
3	56	F	14	Face, scalp	Arms	—	Hydroxychloroquine
4	58	F	11/12	Face, scalp	Arms	—	Intralesional triamcinolone acetonide
5	48	F	6/12	Face, arms	Arms	—	Hydroxychloroquine
6	38	F	2/12	Face	Arms	+	—
7	59	F	4/12	Face	Arms	±	—

plaques. Laboratory examinations including SMA-6,† SMA-12, urinalysis, total haemolytic complement, blood count and differential white blood cell count were within normal limits. LE cell preparation was negative three times. The ANA was negative, and serology tests for syphilis were non-reactive.

† SMA-6 and SMA-12 are automated laboratory analyses for assay of the following constituents in the blood:

SMA-6: Sodium, potassium, chloride, CO₂, BUN, glucose.

SMA-12: Creatinine, calcium, phosphorus, total bilirubin, uric acid, cholesterol, SGOT, LDH, CPK, alkaline phosphatase, total protein, albumin.



FIGURE 4. Multiple hyperkeratotic papules on the arm. The clinical appearance has a close resemblance to keratoacanthomas (Case 3).

Previous treatment of the patient's skin disease consisted of various topical fluorinated corticosteroids and oral hydroxychloroquine without significant improvement in the appearance of either the facial lesions or the lesions on the upper extremities. Previously, a number of hypertrophic lesions on the arms had been removed by surgical excision or cryosurgery, leaving large atrophic scars. Initiation of intralesional injections of approximately 0.1–0.3 ml of triamcinolone acetonide (10 mg/ml) per lesion every 3 weeks has resulted in a gradual involution of the lesions.

Case 2. A 45-year-old male with a 15 year history of discoid LE involving the face was seen at the out-patient clinic of Barnes Hospital. Physical examination revealed a clinical picture strikingly similar to Case 1. On the face, chest and extensor surfaces of the arms, the patient had erythematous lesions demonstrating atrophy, scarring, telangiectasia and scaling (Fig. 2). On his arms, particularly on the wrists and the dorsa of hands, were numerous verrucous papules measuring approximately 1.0–1.5 cm (Fig. 3). Many of the lesions demonstrated a central keratinous plug. The laboratory data including SMA-6, SMA-12, urinalysis and blood count were within normal limits, and the ANA was negative.

Previous treatment consisted of topical corticosteroid preparations without significant improvement and cryosurgery which resulted in large atrophic scars. Biopsies were obtained at the time of the initial visit, but the patient has failed to return for follow-up.

Case 3. A 56-year-old female with a 14 year history of discoid LE has been followed for 2 years in Barnes Hospital out-patient clinic. On the face and scalp were large atrophic areas characterized by erythema, scaling and follicular plugging. Involvement of the scalp has resulted in severe scarring alopecia. On both arms were six hyperkeratotic nodular lesions, 1–3 cm in diameter, surrounded by erythema and marked hyperpigmentation (Fig. 4). Two of the lesions also demonstrated a hard, central keratinous plug.

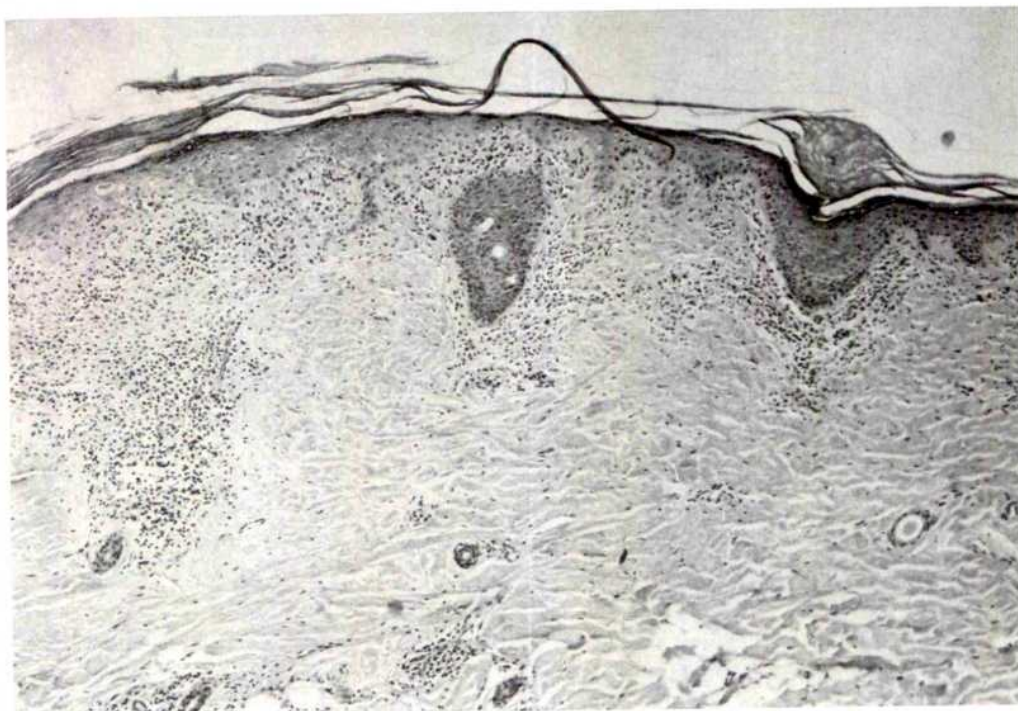


FIGURE 5. The atrophic lesions on the face. A 'cell-poor' lichenoid reaction is present. Note the cytoid bodies in the upper dermis (H & E, $\times 90$).

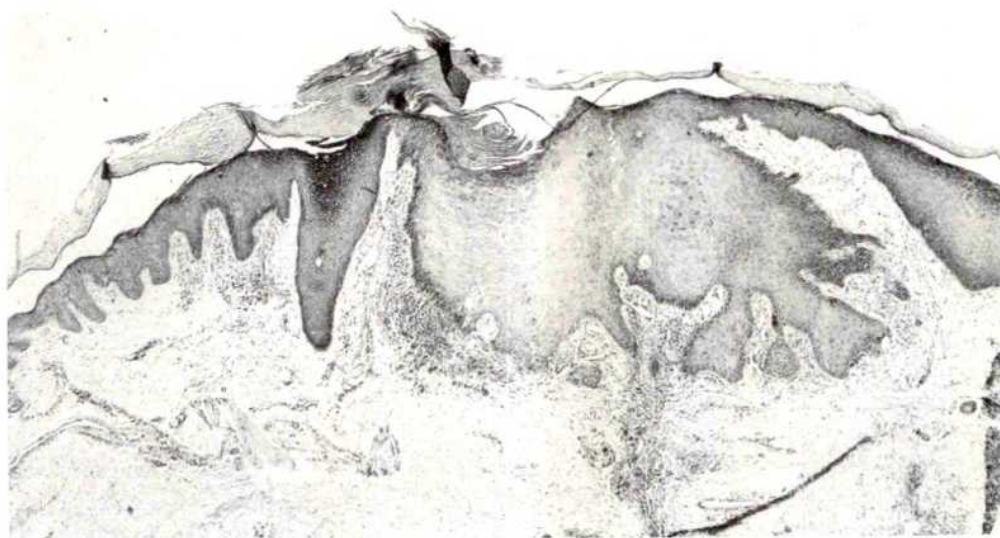


FIGURE 6. Histology of an early hyperkeratotic lesion on the arm. The epidermis has marked acanthosis and hyperkeratosis, and there is a pronounced inflammatory cell infiltrate in the upper dermis (H & E, $\times 40$).



FIGURE 7. Histology of a flat-topped, hyperkeratotic papule on the arm. Two distinct centres of lichenoid reaction are seen with marked hyperkeratosis and moderate acanthosis (H & E, $\times 9.5$).

On several different occasions, the blood count, urinalysis and SMA-12 were within normal limits and the ANA was negative.

Previous treatment consisted of topical fluorinated corticosteroids with minimal improvement. During the past year she has had three 10-week courses of oral hydroxychloroquine (200 mg/day) which resulted in improvement of the scalp and facial lesions but had no effect on the appearance of the arm lesions.



FIGURE 8. A detail of Fig. 7. There is hyperkeratosis with hypergranulosis and a 'cell-rich' infiltrate penetrating the lower portion of the epidermis. The picture has a striking resemblance to lichen planus (H & E, $\times 90$).



FIGURE 9. Histology of a fully developed hyperkeratotic lesion on the arm. The lesion has a striking resemblance to keratoacanthoma (H & E, $\times 12$)

Case 4. A 58-year-old female with an 11 month history of skin lesions was referred by a local dermatologist. On the first visit lesions typical of discoid LE were present on the face and scalp. On both arms there were several non-pruritic hyperkeratotic raised 1–3 cm papulo-nodular lesions, which showed no tendency to coalesce. The arm lesions appeared at about the same time as the facial lesions. Physical examination was otherwise unremarkable.

The laboratory tests showed normal protein electrophoresis, negative rheumatoid factor and negative ANA. The sedimentation rate was 34 mm/h (normal limits 0–20). SMA-12, total haemolytic complement and urinalysis were within normal limits. Initial therapy by the referring physician consisted of a short course of oral prednisone and topical fluorinated corticosteroids without noticeable

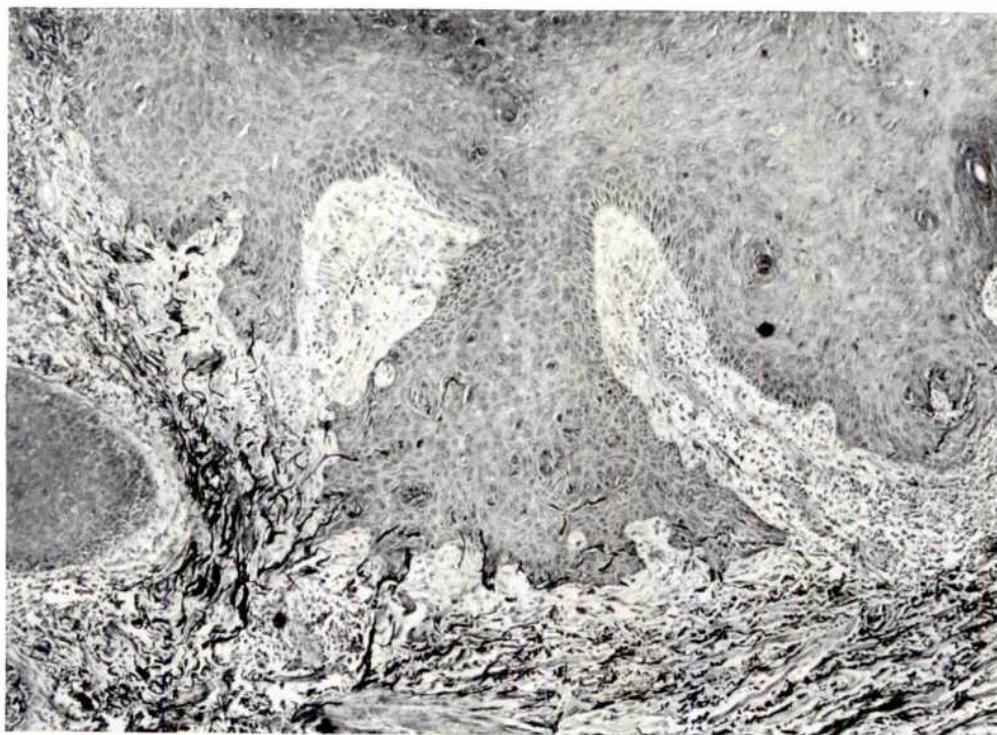


FIGURE 10. A detail of Fig. 9. Elastic fibres are present in the upper dermis and the epidermis (Iron-Gallein, $\times 90$).



FIGURE 11. Immunofluorescent staining of a papular lesion on the arm. Brightly staining globular bodies at the dermo-epidermal junction and a discontinuous staining at the basement membrane zone on the right-hand side of the picture are seen (anti-human IgG).

improvement. Intralesional injection of triamcinolone acetonide, 10 mg/ml, was initiated and has led to a gradual regression of the verrucous lesions.

Case 5. A 48-year-old female was referred by a local dermatologist because of a 6 month history of skin lesions on face, back and arms. Physical examination revealed typical discoid LE involving these areas. In addition, the patient had raised verrucous papules with a central keratinous plug on the arms. The ANA was negative. To date the patient has received oral hydroxychloroquine with a moderate improvement of the atrophic, erythematous lesions.

Case 6. A 38-year-old female with about a 2 month history of skin lesions was seen at the out-patient clinic of Barnes Hospital in March 1977. On the face the patient had bilateral lesions characterized by atrophy, telangiectasia and scaling. On both arms were three isolated lesions of about 2–3 cm in

diameter. These lesions were raised, hyperkeratotic and surrounded by marked erythema. Laboratory tests including blood count, urinalysis and SMA-12 were within normal limits, except for an elevated CPK of 390 units. The ANA was positive, 3+ in 1:10 dilution, with a homogeneous nuclear staining pattern. LE cell preparation was negative. The physical examination was otherwise unremarkable.

Case 7. A 59-year-old female was referred by a local dermatologist because of a 4 month history of lesions on the face and arms. On the face were two erythematous lesions characterized by atrophy, telangiectasia and scaling, and on the arms were three raised erythematous papules, one of which had a central keratinous plug. Laboratory tests including SMA-12, urinalysis, blood count and total haemolytic complement were within normal limits. The ANA was reported as trace positive. The physical examination was otherwise unremarkable.

HISTOLOGY

Although considerable histological variation was seen in the skin lesions, they can be divided basically into two groups which correspond to the clinical picture: (a) the classical atrophic lesions of discoid LE; and (b) the verrucous papulo-nodular lesions.

Biopsies from the classical areas of discoid LE were taken to confirm the clinical diagnosis (Fig. 5). In these cases the epidermis was atrophic, having only 4 or 5 layers of keratinocytes, and there was mild hyperkeratosis with follicular plugging. The lesions also demonstrated variable vacuolization of the basal layer. Scattered mononuclear cells were invading the epidermis, and in these areas the basement membrane was either markedly thickened or normal. The thickening was more evident in sections stained with PAS. The dermis often showed foci of actinic damage as well as evidence of delicate

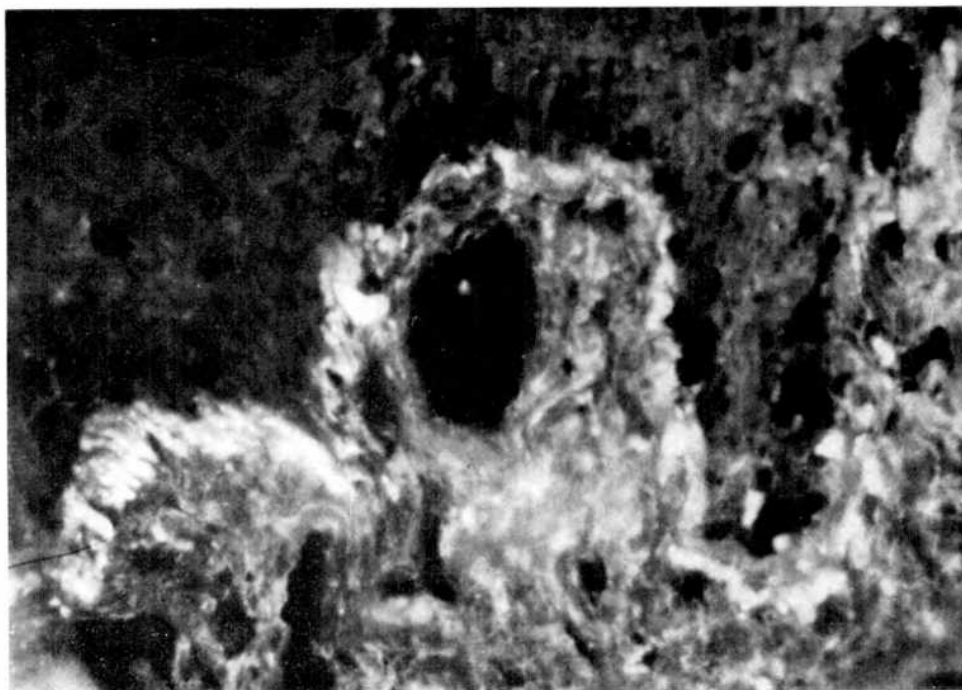


FIGURE 12. Granular deposition of IgG along the basement membrane zone (anti-human IgG).

TABLE 2. Immunofluorescence studies on atrophic and verrucous skin lesions

Case no.	Type of lesion	Basement membrane zone					Upper dermis				
		IgG	IgA	IgM	Deposition of complement	Fibrinogen	IgG	IgA	IgM	Deposition of complement	Fibrinogen
1	{ Atrophic	+†			+						
	{ Verrucous	+	+		+		+	+	+		
3	{ Atrophic	+	+	+	+	+			+		
	{ Verrucous	+	+	+	+	+	+	+	+	+	+
4	{ Atrophic	+			+						
	{ Verrucous	+		+			+		+		
6	Verrucous	+		+	+		+		+	+	
7	Verrucous	+		+			+		+	+	

* The numbers refer to the case reports in the text.

† + Indicates the presence of a specific deposition.

fibrosis. The vessels appeared dilated with thin walls but occasionally capillaries with a thickened eosinophilic wall were seen. An inflammatory cell infiltrate was located primarily in the superficial dermis and showed a tendency to concentrate around blood vessels and hair follicles. The cellular infiltrate was composed mainly of lymphocytes, but histiocytes, rare plasma cells and numerous macrophages with phagocytized melanin granules were also seen. Hyaline epidermal bodies, cytoid or Civatte bodies (Ueki, 1969) were present in older lesions and they sometimes alternated with vacuolar changes of the basal keratinocytes (Fig. 5).

The histology of the specimens taken from the papulo-nodular, verrucous lesions appeared to be dependent on the age of the lesions. The early lesions showed a moderate degree of acanthosis with irregular elongation of the rete pegs (Figs 6 & 7). Hyperkeratosis associated with a very thick granular layer, occasionally composed of 5 or 6 rows of cells, was present (Fig. 8). In the superficial dermis a band-like mononuclear cellular infiltrate was present which tended to hug the epidermis, sometimes erasing the dermo-epidermal junction (Fig. 8). Numerous cytoid bodies were seen in a linear arrangement along the epidermis. Older lesions demonstrated progressive fibrosis and vascular proliferation. A focally thickened basement membrane was present, and lymphocytes were observed in the deeper dermis (Fig. 9). Curly elastic fibres as well as amorphous elastic material were engulfed by the epithelial islands (Fig. 10). This material frequently extended upwards into the epidermis. This phenomenon was particularly evident in sections stained for elastic tissue and was most prevalent when there was acanthosis of the epidermis. The verrucoid lesions extended laterally in a plaque-like appearance (Fig. 7) or had a localized invasive growth in a tumour-like fashion (Figs 6 & 9). The plaque-like lesions had marked hyperkeratosis and hypergranulosis. The acanthosis was mostly regular and the lymphocytic infiltrate was dense in a band-like distribution (Figs 10 & 11). The similarity of this process to hypertrophic lichen planus was remarkable.

In contrast, the tumour-like lesions adopted a cup-shaped growth with a central crater full of keratin (Figs 7 & 8). The deep margins, however, still preserved the lichenoid character. The keratinocytes had a glassy appearance and the elastic tissue engulfed by the tumour was more prominent than in the more superficial lesions. The histology was indistinguishable both architecturally and cytologically from that of a keratoacanthoma.

IMMUNOFLUORESCENCE

Three patients had immunofluorescent studies of the lesions considered classical for discoid LE. Biopsy specimens from the verrucous lesions in five patients were also examined for direct immunofluorescence. The findings are summarized in Table 2. In typical discoid LE, IgG and C₃ were positive at the basement membrane zone of the epidermis in a linear pattern and on occasion, in the upper portion of the hair follicles. In the verrucous, papulo-nodular lesions, there was focal staining for IgG in a granular pattern at the basement membrane zone (Figs 11 & 12). To a lesser degree, IgM and C₃ were deposited along the basement membrane. Large clumps of immunoglobulin were also found in the superficial dermis immediately adjacent to the epidermis; these globules were principally IgM, but they also stained with anti-IgA and anti-IgG. Autofluorescent elastic fibres were seen at different levels in the epithelial islands.

COMMENT

Discoid LE frequently presents a spectrum of cutaneous findings. Most commonly the lesions are localized to the sun-exposed areas, but a disseminated form of the disease also occurs. In both instances, however, the individual lesions have the same clinical features. One variant of discoid LE, termed lupus erythematosus hypertrophicus by Bechet (1942), is characterized by raised, erythematous and hyperkeratotic plaques. The predilection sites are the lower lip, sides of mouth and the chin. In another variant of lupus erythematosus, lupus profundus (Kaposi and Irgang), deep cutaneous nodules are seen which are often associated with the usual discoid variety of lupus erythematosus. In lupus profundus, the subdermal fat is occasionally involved, and the lesions eventually heal with deep atrophy of the involved area.

Of particular interest is the association of less clearly defined lesions in patients also having the characteristic features of typical discoid LE. A number of reports have described cases which have clinical, histological and immunofluorescent features of both lupus erythematosus and lichen planus (Copeman *et al.*, 1970; Thorman, 1974; Umbert *et al.*, 1974; Davies *et al.*, 1977; Romero *et al.*, 1977). In the cases presented by Romero *et al.* (1977) the lesions were characterized by marked atrophy and the acral aspects of both upper and lower extremities were most commonly affected. On the basis of the clinical and immunological findings the authors felt that a definitive diagnosis of either lichen planus or lupus erythematosus could not be made, and they suggested that some of the patients might possibly have both diseases.

In the present study we describe seven patients all having typical lesions of discoid LE on the face and scalp. The histology of the skin biopsies taken from these areas demonstrated the classical changes of discoid LE namely vacuolization of the basal layer, thickening of the basal lamina and the linear deposition of immunoglobulins and complement at the basement membrane zone. In addition, all seven patients demonstrated verrucous lesions mainly on the upper extremities. The clinical picture of these lesions was highly variable. Some were characterized by marked hyperkeratosis with features of lichen planus. These cases, however, were clearly different from those presented by Romero *et al.* (1977) in that there was no atrophy, and no oral or nail involvement was seen. Remarkably, the lesions were non-pruritic. Some of the lesions resembled prurigo nodularis (Kocsard *et al.*, 1972) or multiple acral fibrokeratomas (Reed & Elmer, 1971). Others presented as papulo-nodular lesions with a keratinous centre, a picture clinically indistinguishable from multiple keratoacanthomas or self-healing squamous cell epithelioma (Ayres, 1949; Epstein *et al.*, 1957; Ereaux & Schopflocker, 1965).

Previously, an association between lupus erythematosus and keratoacanthoma has been suggested

(Jolly & Carpenter, 1966). In particular, a group of patients originally described by Poth (1939) demonstrated pronounced light sensitivity and subsequent production of tumour-like keratoses (New & Horton, 1953; Fliegelman & Loveman, 1957), features of the patients described here.

The histology of the verrucous lesions from our patients, although variable, showed a remarkable similarity to keratoacanthoma. In particular, the lesions frequently demonstrated a shallow crateriform keratinous centre, a characteristic feature observed in the early stages of keratoacanthoma. However, the marked cup formation which occurs during the maturation of a keratoacanthoma was not seen. Some of the lesions also had a histological picture resembling lichen planus. Certain features, however, were helpful in distinguishing these two conditions. A consistent observation was the presence of elastic fibres in the acanthotic epidermis. Although the elastic material had a tendency to stay in the basal portions of the epidermis, some of the fibres were also seen in the upper part of the epidermis and within the horny layer. This phenomenon is frequently seen in keratoacanthoma and discoid LE but has not been encountered in lichen planus (Mehregan, 1970).

The immunofluorescent findings were characteristic, although not diagnostic, of discoid LE. The presence of immunoglobulins in a globular pattern in the dermis with occasional basement membrane staining has been described in several disorders (Ueki, 1969), among them keratoacanthoma (Ramseelaar & van der Meer, 1976), lupus erythematosus and lichen planus (Beutner *et al.*, 1973). This immunofluorescent pattern as such has no diagnostic significance, and may be representative of the lichenoid reaction. This pattern when seen in keratoacanthoma is perhaps the result of an epithelial-stromal interaction, and its presence may be related to the immunological aspects of tumour rejection (Brown & Tan, 1973). As mentioned above the verrucous lesions occasionally had clinical features reminiscent of prurigo nodularis or multiple acral fibrokeratomas. These two conditions, however, lack the lichenoid reaction.

Of particular interest in evaluating the relationship between discoid LE and lichen planus is a recent study by Davies & Marks (1977) on the processes leading to the development of epidermal changes in discoid LE. Their results suggest that in discoid LE a very active repair mechanism in the epidermis is operative, a finding also noted in their previous studies of lichen planus (Marks *et al.*, 1973; Presbury & Marks, 1974). The authors suggested that differential deposition of immunoglobulins at the dermo-epidermal junction could partly explain the differences in the overall morphology of discoid LE and lichen planus.

The clinical course of the verrucous lesions was characterized by marked chronicity with progressive involvement of the hands and arms. Spontaneous regression of the skin lesions has not, as yet, been seen in any of the patients. Furthermore, treatment is difficult and often unrewarding. The topical application of various fluorinated corticosteroid preparations, 5-fluorouracil or retinoic acid produced no effect. A short course of oral prednisone in one of the patients was not beneficial. Two of the patients received oral hydroxychloroquine for various time periods without improvement. The most effective treatment modality consisted of intralesional injections of triamcinolone acetonide (10 mg/ml 0.2–0.4 ml per lesion). When injected at 2–3 week intervals, the verrucous lesions became less infiltrated and hyperkeratotic. The results of prolonged intralesional steroid therapy are still under evaluation.

The patients presented in this study resemble each other in that they have typical atrophic discoid LE lesions on the face and/or scalp and multiple verrucous lesions principally localized to the upper extremities. Because of the close resemblance of the clinical picture in all seven cases, it appears unlikely that the existence of the two different types of lesions in the same patient is purely coincidental. Even though the verrucous lesions have both clinical and histological findings in common with keratoacanthoma and lichen planus, features are present which suggest that they represent an unusual and previously unrecognized form of discoid LE.

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REFERENCES

- AYRES, S. (1948) Squamous cell epithelioma (self-healing type). *Archives of Dermatology*, **58**, 584.
- BEUTNER, E.H., CHORZELSKI, T.P., BEAN, S.F. & JORDAN, R.E. (1973) *Immunopathology of the Skin. Labeled Antibody Studies*. Dowden, Hutchinson & Ross, Inc., Strausbury, Pa. U.S.A.
- BECHET, P.E. (1940) Hypertrophic lupus erythematosus. *Archives of Dermatology and Syphilology*, **42**, 211.
- BECHET, P.E. (1942) Lupus hypertrophicus et profundus. *Archives of Dermatology*, **45**, 33.
- BECHET, P.E. (1950) Lupus erythematosus hypertrophicus et profundus: A further attempt to elucidate its status. *Archives of Dermatology*, **61**, 495.
- BROWN, F.C. & TAN, E.M. (1973) Immunological reaction in keratoacanthoma, a spontaneously resolving skin tumor. *Cancer Research*, **33**, 2030.
- COPEMAN, P.W.M., SCHROETER, A.L. & KIERLAND, R.R. (1970) An unusual variant of lupus erythematosus or lichen planus. *British Journal of Dermatology*, **83**, 269.
- DAVIES, M.G. & MARKS, R. (1977) Evolution of the lesion of discoid lupus erythematosus; a comparison with lichen planus. *British Journal of Dermatology*, **97**, 313.
- DAVIES, M.G., GORKIEWICZ, A., KNIGHT, A. & MARKS, R. (1977) Is there a relationship between lupus erythematosus and lichen planus? *British Journal of Dermatology*, **96**, 145.
- EPSTEIN, N.N., BISKIND, G.R. & POLLACK, R.S. (1957) Multiple primary self-healing squamous-cell 'epitheliomas' of the skin. *Archives of Dermatology*, **75**, 210.
- EREAUX, L.P. & SCHOPFLOCKER, P. (1965) Familial primary self-healing squamous epithelioma of skin. Ferguson-Smith type. *Archives of Dermatology*, **91**, 589.
- FEVERMAN, E.J. & HALEVY, S. (1977) Lupus erythematosus profundus (Kaposi-Irgang) with monoclonal gammopathy. *British Journal of Dermatology*, **96**, 79.
- FLIEGELMAN, M.T. & LOVEMAN, A.B. (1957) Tumor-like keratoses. Report of a case. *Archives of Dermatology and Syphilology*, **66**, 353.
- IRGANG, S. (1940) Lupus erythematosus profundus. *Archives of Dermatology and Syphilology*, **42**, 97.
- JOLLY, H.W., Jr & CARPENTER, C.L., Jr (1966) Multiple keratoacanthomata. A report of two cases. *Archives of Dermatology*, **93**, 348.
- KAPOSI, M. (1883) *Pathologie und Therapie der Hautkrankheiten*, 2nd ed, p. 642. Urban & Schwarzenberg, Vienna.
- KOCSARD, E., PALMER, G. & CONSTANCE, T.J. (1972) Multiple keratoacanthoma-like neurodermatitis nodularis. *Australasian Journal of Dermatology*, **13**, 100.
- MARKS, R., BLACK, M.M. & WILSON JONES, E. (1973) Epidermal cell kinetics in lichen planus. *British Journal of Dermatology*, **88**, 37.
- MEHREGAN, A.H. (1970) Transepithelial elimination. *Current Problems in Dermatology*, **3**, 124.
- NEW, W.M. & HORTON, C.S.H. (1953) Multiple acanthoma (tumor-like keratosis of Poth). *Archives of Dermatology and Syphilology*, **68**, 475.
- OTANI, A. (1977) Lupus erythematosus hypertrophicus et profundus. *British Journal of Dermatology*, **96**, 75.
- POTH, D.O. (1939) Tumor-like keratoses: Report of a case. *Archives of Dermatology and Syphilology*, **39**, 228.
- PRESBURY, D.G.C. & MARKS, R. (1974) The epidermal disorder of lichen planus: An *in vitro* study. *British Journal of Dermatology*, **90**, 373.
- RAMSELAAR, C.G. & VAN DER MEER, J.B. (1976) The spontaneous regression of keratoacanthoma in man. *Acta dermato-venerologica*, **56**, 245.
- REED, R.J. & ELMER, L.C. (1971) Multiple acral fibrokeratomas (a variant of prurigo nodularis). *Archives of Dermatology*, **103**, 286.
- ROMERO, R.W., NESBITT, L.T. & REED, R.J. (1977) Unusual variant of lupus erythematosus or lichen planus. *Archives of Dermatology*, **113**, 741.
- THORMANN, J. (1976) Ulcerative lichen planus of the feet. A case in which the serological findings suggested systemic lupus erythematosus. *Archives of Dermatology*, **110**, 753.
- TUFFANELLI, D.L. (1972) Lupus erythematosus. *Archives of Dermatology*, **106**, 553.
- UEKI, H. (1969) Hyaline bodies in subepidermal papillae. *Archives of Dermatology*, **100**, 610.
- UMBERT, P., DUPERRAT, B., PUISSANT, A., SAURAT, J.H. & PACOT, Cl. (1974) Lichen verruqueux. Lupus érythémateux. Études clinique, histologique et immunofluorescence. *Bulletin de Société Française de Dermatologie et de Syphiligraphie*, **81**, 467.

Lymphocyte subpopulations, serum immunoglobulins and complement factors in patients with atopic dermatitis

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SUMMARY

The percentages of lymphocytes carrying different classes of membrane-bound Ig, and lymphocytes forming rosettes with sheep erythrocytes (E-RFC), as well as lymphocytes with receptor for the Fc-part of IgG (EA-RFC) were determined in 19 patients with atopic dermatitis. Lymphocyte suspensions were also stained with a specific rabbit anti-human T-lymphocyte antiserum. Furthermore, the serum concentration of IgG, IgA, IgM and IgE and the complement factors C3 and C4 were measured. A small but significant increase in lymphocytes with membrane-bound IgE and an increase in the serum concentration of IgE and complement factor C4 were observed. A decrease in the percentages of lymphocytes with receptors for sheep erythrocytes (E-RFC) was also found. The percentages of lymphocytes that stained with the anti-T antiserum correlated well with the percentages of lymphocytes forming rosettes with sheep erythrocytes. In one patient we found increased IgE positive lymphocytes, increased IgE serum concentration and a decreased T-cell number.

A previous study revealed that the percentage of peripheral lymphocytes with membrane-bound IgE is considerably raised in patients with atopic dermatitis (Cormane, Husz & Hamerlinck, 1974). Other investigators have observed a slightly increased percentage of IgE bearing lymphocytes in these patients (Carapeto, Winkelmann & Jordon, 1976; Hovmark, 1977). A decrease of lymphocytes with receptors for sheep erythrocytes, T-lymphocytes, has also been reported (Andersen & Hjorth, 1975; Hanifin, 1974; Luckasen *et al.*, 1974; Strannegård, Lindholm & Strannegård, 1976) as well as decreased cell-mediated immunity (Rajka, 1968; Hovmark, 1975; Lobitz, Honeyman & Winkler, 1972; Strannegård *et al.*, 1976; McGeady & Buckley, 1975). Several authors have found an increase in the serum concentration of IgE (Cohen, 1976; Ogawa *et al.*, 1971) while the other immunoglobulin

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classes usually have normal values. We now report features of the cellular and humoral immunity in patients with atopic dermatitis. IgE bearing lymphocytes were specially examined because of earlier inconstant findings and a recent report on human lymphocytes with receptor for IgE (Gonzales-Molina & Spiegelberg, 1977).

MATERIALS AND METHODS

Patients. Nineteen patients in hospital with the diagnosis of atopic dermatitis, 11 females and 8 males with a mean age of 22 years, range 5–53 years, and a mean duration of disease of 20 years, range 2–52 years, were included. There were 11 healthy controls, 8 females and 3 males, mean age 31, range 22–55. All the patients had a personal history of atopy, and most both a personal and family history of atopy. None were treated during the week before investigation. Blood was drawn and separated by the Isopaque–Ficoll technique (Bøyum, 1976).

Identification of various lymphocyte populations

B-lymphocytes. Membrane bound Ig as determined by the immunofluorescence technique was used as a marker of human B-lymphocytes. The various lymphocyte suspensions were stained with anti-F(ab')₂ and anti-Ig class antisera (Frøland & Natvig, 1972, 1973).

T-lymphocytes. T-lymphocytes with surface receptors for sheep erythrocytes were detected with a rosette-technique (Frøland, 1972; Frøland & Natvig, 1973).

EA-rosette forming cells. Rosette forming cells were detected after reaction of lymphocytes with human-IgG sensitized erythrocytes as described elsewhere (Frøland & Natvig, 1973).

Antisera

Rabbit anti-human T-lymphocyte antiserum. Thymocytes were extracted from pieces of thymus obtained from children undergoing cardiac surgery. After isolation, the thymocyte suspensions were used to immunize rabbits. The rabbit anti-human thymocyte antiserum was rendered T-lymphocyte specific by extensive absorptions with AB and O erythrocytes, pooled human IgG, and B-lymphocytes from patients with chronic lymphocytic leukaemias (paper in preparation). The anti-T-lymphocyte antiserum was then used in an indirect immunofluorescence technique with a fluoresceinated goat anti-rabbit antiserum as the second layer.

Anti-Ig class and anti-complement antisera. Antisera against different Ig classes and IgG F(ab')₂ fragments were raised in rabbits while antisera against C₃ and C₄ were obtained from Behringwerke (Behringwerke AG, Marburg-Lahn, W. Germany).

Determination of serum immunoglobulins and complement factors. IgG, IgA, IgM, C₃ and C₄ were measured by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965). The concentration of serum IgE was measured by means of radioimmunoassay utilizing Pharmacia Diagnostics Phadebas IgE test.

Immunofluorescence staining and microscopy

Direct immunofluorescence staining was performed with all antisera except the anti-T-lymphocyte antiserum. Briefly 5×10^6 lymphocytes in medium 199 (Grand Island Biological Co., Grand Island,

New York) were incubated with 0.1 ml of FITC-conjugated antisera for 20 min at 4°C. After incubation the cells were washed once in Hanks' BSS (Grand Island Biological Co., Grand Island, N.Y.), and once in McCoy's 5A medium (Gibco, Bio-Cult, Glasgow, Scotland). Immunofluorescence staining with antiserum against the thymocytes was performed by the indirect immunofluorescence technique. After incubation with the unconjugated antiserum for 20 min at 4°C the cells were washed twice in medium 199 before FITC conjugated goat anti-rabbit immunoglobulin antiserum (Behringwerke AG, Marburg-Lahn, W. Germany) was added. After incubation for 20 min at 4°C, the same washing procedure was performed as described for the direct immunofluorescence method. A Leitz Orthoplan Microscope with an Osram HBO-200 high pressure Mercury Vapor-burner light source and a Leitz vertical illuminator was used as described in detail previously (Frøland & Natvig, 1972b). Lymphocytes were examined with an oil immersion ($\times 54$) objective. A minimum of 200 cells per preparation were counted. Monocytes and granulocytes were excluded as judged by the size, the intracellular staining, and the shape of the nucleus.

Statistical methods. Wilcoxon's two-sample test, and Student's 't'-test as a control.

RESULTS

Lymphocytes with membrane bound Ig

Immunofluorescence staining was performed with FITC-conjugated anti-Ig class and anti-F(ab')₂ antisera on Isopaque-Ficoll separated cells from 19 patients with atopic dermatitis and from 11 healthy controls. The percentages of lymphocytes with membrane-bound IgE were increased to 2% in the patient group compared with the normal controls who had <1% IgE positive cells ($P < 0.01$) (Fig. 1). On the other hand, no statistical differences in the percentages of lymphocytes positive with anti-F(ab')₂, anti-IgG, anti-IgA, anti-IgM and anti-IgD between the two groups were observed (Table 1). The sum of the percentages of lymphocytes positive with anti-class antisera always equalled the percentage of lymphocytes positive with the anti-F(ab')₂ antiserum.

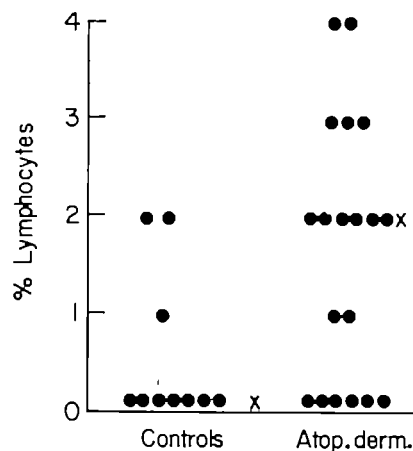


FIGURE 1. Scattergram showing the percentages of IgE-positive lymphocytes in patients with atopic dermatitis and healthy controls. The difference is significant, $P < 0.01$. X=median.

TABLE 1. Comparison of the percentages of lymphocytes positive with various anti-class and anti-F(ab')₂ antisera in patients with atopic dermatitis and healthy controls

	Atop. derm.		Controls		Test for difference atop. derm. vs controls
	Mean	s.d.	Mean	s.d.	
ly a-F(ab') ₂	14.5	5.4	13	3.7	n.s.
ly-IgG	4.9	2.2	3.9	1.3	$P < 0.1$
ly-IgA	2.1	1.3	2.3	1.1	n.s.
ly-IgM	6.1	2.6	6.4	1.1	n.s.
ly-IgD	3.8	1.9	4.5	1.1	n.s.

n.s., Not significant.

TABLE 2. Comparison of the percentages of lymphocytes positive with the anti-T-antiserum and the percentages of lymphocytes forming rosettes with sheep erythrocytes in patients with atopic dermatitis and healthy controls

	E-RFC(%) Anti-T(%)	
Patient No.		
1	44	46
2	57	59
3	45	46
4	41	45
5	37	41
Control No.		
1	54	56
2	57	58
3	56	58
4	60	60
5	64	65

Lymphocytes with receptors for sheep erythrocytes (E-RFC) and with receptors for the Fc part of human IgG (EA-RFC)

Rosettes with sheep erythrocytes and with IgG (anti-C+D) coated human group OR₁R₂ red cells were made as mentioned above. The mean percentage of E-RFC, T-lymphocytes, was 51.5% and thus lower in the patient group than in the control group with 57.4% E-RFC ($P < 0.05$) (Fig. 2). No difference between the two groups, however, was observed for the mean percentage of EA-RFC (Fig. 3).

The numbers of lymphocytes that formed rosettes with sheep erythrocytes were of the same magnitude as the percentages of lymphocytes positive with the anti-T antiserum (Table 2).

The concentrations of serum immunoglobulins and complement factors

The serum immunoglobulin concentrations of the classes IgG, IgA and IgM, and the concentrations of C₃ and C₄ complement factors were measured by means of immunodiffusion, while serum IgE was measured by radioimmunoassay. The concentration of serum IgE had significantly higher values in the patient group with 715 u/ml than in the control group with 80 u/ml ($P < 0.01$) (Fig. 4). The same tendency was observed for the complement factor C₄ with a concentration of 0.37 g/l in the patient group and 0.21 g/l in the control group ($P < 0.01$) (Fig. 5). No differences in the concentrations of C₃ complement factor and the other immunoglobulin classes were observed between the two groups (Table 3).

The effect of incubation on membrane-bound immunoglobulins

To investigate whether the immunoglobulin molecules demonstrated on the lymphocytes were passively adsorbed or actively produced by the cells, some incubation experiments were performed. Lymphocytes isolated from 5 patients with atopic dermatitis were stained before and after incubation

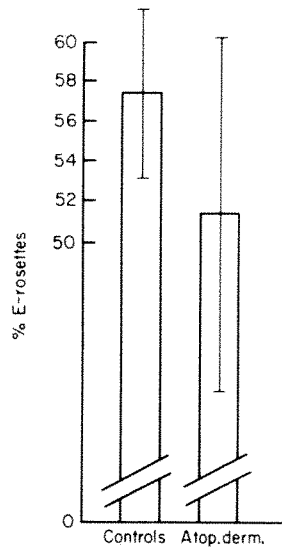


Fig. 2.

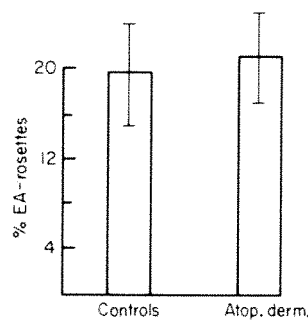


Fig. 3.

FIGURE 2. Difference between the mean percentage of cells forming rosettes with sheep red blood cells in 19 patients with atopic dermatitis and 11 healthy controls. The difference is significant, $P < 0.05$.

FIGURE 3. Percentage of lymphocytes forming rosettes with IgG (Anti-C+D) coated human group OR₁R₂ red cells in 19 patients with atopic dermatitis and 11 healthy controls. No significant difference was observed.

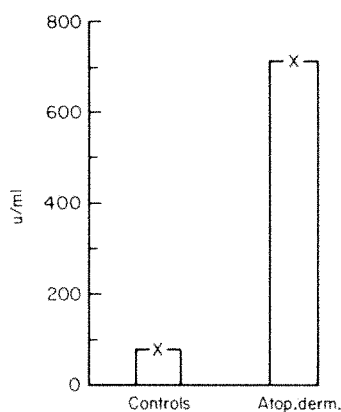


Fig. 4.

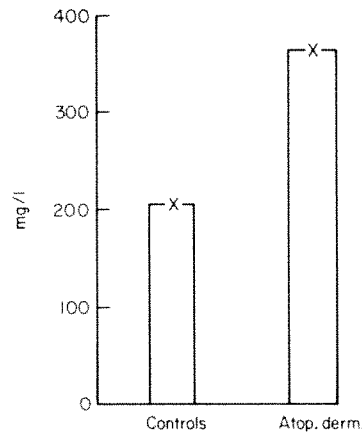


Fig. 5.

FIGURE 4. Quantitative difference in serum IgE in 16 patients with atopic dermatitis and 11 healthy controls. The difference is significant, $P < 0.01$. X=median.

FIGURE 5. Quantitative difference in serum C4 in 15 patients with atopic dermatitis and 11 healthy controls. The difference is significant, $P < 0.01$. X=median.

at 4°C for 24 h, at 37°C for 24 h and at 37°C for 48 h in McCoy's 5A medium with 20% fetal calf serum (Grand Island Biological Company, Grand Island, New York). The percentage of IgE-positive lymphocytes decreased from a mean of 2.08% to a mean of 1.60% during the incubation. Similarly, a small fall in the mean percentage of Ig-positive cells from 14.0% to 12.4% was observed with the same incubation procedure (Table 4).

TABLE 3. Serum concentrations of IgG, IgA, IgM and C3 in patients with atopic dermatitis and healthy controls (g/l)

	Atopic dermatitis		Healthy controls		Test for diff. atop. dermat. vs controls
	Mean	s.d.	Mean	s.d.	
IgG	13.6	4.2	12.2	2.6	n.s.
IgA	1.8	0.9	1.8	0.9	n.s.
IgM	1.5	0.8	1.6	0.6	n.s.
C3	0.95	0.18	0.7	0.1	n.s.

n.s., Not significant.

TABLE 4. Percentages of lymphocytes staining with anti-IgE and anti-F(ab')₂ antisera from 5 patients with atopic dermatitis before and after incubation in McCoy's Medium at different temperatures and periods of time

	Before incubation (%)		After 24 h at +4°C (%)		After 24 h at 37°C, 5% CO ₂ (%)		After 48 h at 37°C, 5% CO ₂ (%)	
	Patient anti-F(ab') ₂	anti-IgE	anti-F(ab') ₂	anti-IgE	anti-F(ab') ₂	anti-IgE	anti-F(ab') ₂	anti-IgE
a	14.2	2.2	11.4	2.6	10.0	2.4	12.2	2.0
b	11.0	2.4	12.8	1.8	11.6	2.2	10.4	1.2
c	13.6	1.0	10.8	1.4	12.0	1.0	9.4	0.8
d	15.0	1.8	11.6	2.2	9.8	2.0	12.2	1.6
e	16.2	3.0	18.0	2.6	19.6	2.2	17.8	2.4
Mean	14.0	2.08	12.92	2.12	12.6	1.96	12.4	1.60

DISCUSSION

In the present study a moderate, but significant increase in the mean of the percentages of the lymphocytes with membrane-bound IgE was demonstrated in a group of patients with atopic dermatitis as compared with a control group of healthy persons. These findings are in accordance with other reports (Cormane *et al.*, 1974; Carapeto *et al.*, 1976; Hovmark, 1977). We found a mean percentage of 2% IgE positive lymphocytes; Hovmark found that most patients had less than 3% of IgE positive cells. In other studies a mean of 8.43% (Carapeto *et al.*, 1976) and a mean of 20.5% IgE positive lymphocytes (Cormane *et al.*, 1974) were reported. These differences are difficult to explain, but there are several pitfalls in the demonstration of lymphocytes with true membrane-bound IgE molecules: In patients with high serum concentrations of IgE, IgE molecules might adsorb to the membrane of the

lymphocytes and basophil granulocytes that have receptors for the Fc part of IgE (Gonzales-Molina & Spiegelberg, 1977; King, Alaye & Augustin, 1976).

In the present paper we tried to avoid this pitfall in two ways. Only cells judged to be lymphocytes with positive membrane fluorescence were counted. We also made some incubation experiments permitting the incubated lymphocytes to elute adsorbed IgE molecules. These experiments indicate that some IgE might have been adsorbed to the lymphocyte membrane, and is shed during the incubation as judged by the fall in the mean percentage of the IgE positive cells. But even this mean percentage of 1.6% IgE positive cells is higher than the mean percentage of the IgE-positive cells in the control group; this indicates an increase in number of lymphocytes actively synthesizing IgE.

When a small percentage of cells, as reported here, show positive staining with anti-IgE antiserum, there may be another pitfall in that the membrane-bound Ig have anti-immunoglobulin activity. This is most likely to happen when the conjugate source is goat, sheep or guinea-pig, since heterophile antibodies to gammaglobulins from these species are often produced in man (Brandtæg & Baklien, 1976). This must also be considered in this work, even when we use conjugated rabbit antibodies towards which heterophile antibodies are seldom directed in man.

The mean percentage of B-lymphocytes in the present study was normal. This is in accordance with other authors (Luckasen *et al.*, 1974; Strannegård *et al.*, 1976; Thestrup-Pedersen *et al.*, 1977; Grove, Reid & Forbes, 1975).

The concentration of serum IgE was significantly higher in the patient group than in the control group, while the concentration of the other immunoglobulin classes had normal values. Similar results have also been demonstrated by others (Grove *et al.*, 1975; Rogge & Hanifin, 1976; Øhman & Johansson, 1974; Slavin, 1976). The increase in the serum IgE level correlates well with a concomitant rise in the percentages of IgE positive lymphocytes in the patient group and which are precursors for mature IgE-producing plasma cells. The increased serum concentration of C4 in the patient group will not be discussed.

A decrease in the mean percentage of peripheral blood T-lymphocytes was demonstrated in the present work, as judged from the percentages of lymphocytes forming rosettes with sheep erythrocytes. Similar results have also been published elsewhere (Luckasen *et al.*, 1974; Carapeto *et al.*, 1976; Strannegård *et al.*, 1976; Thestrup-Pedersen *et al.*, 1977). This finding may parallel a decreased cell mediated immunity observed by others (Lobitz, Honeyman & Winkler, 1972). Thus it has also been proposed that atopic dermatitis results from a defect in the T-cell system or a fall in the number of suppressor T-cells which may lead to a rise in the concentration of serum IgE (Maize, 1976; Rogge & Hanifin, 1976). In a mouse system it has been shown that T-cells and thymocytes have a suppressor effect on IgE production (Watanabe, Kojima & Ovary, 1976). This should therefore be investigated in proper human test systems. Other authors have found a correlation between a decrease in the T-cell percentage and a rise in the concentration of serum IgE (Maize, 1976; Thestrup-Pedersen *et al.*, 1977). Still others have not been able to demonstrate this phenomenon (Andersen & Hjorth, 1975). However, any relationship between a decreased number of T-lymphocytes and suppressor cells on one hand, and an augmentation in the serum IgE on the other hand in man is yet a speculation. Thus only one patient in the present work had a decreased number of T-lymphocytes together with a rise in serum IgE concentration and an increased number of IgE positive lymphocytes. The fall in the percentages of blood T-lymphocytes could also reflect the fact that the lymphocytic, dermal, perivascular infiltrates seen in atopic dermatitis are predominantly T-lymphocytes (paper in preparation).

REFERENCES

- ANDERSEN, E. & HJORTH, N. (1975) B Lymphocytes, T Lymphocytes and phytohaemagglutinin responsiveness in atopic dermatitis. *Acta dermato-venereologica* (Stockholm), **55**, 345.

- BRANDTZAEG, P. & BAKLIEN, K. (1976) Inconclusive immunohistochemistry of human IgE in mucosal pathology. *Lancet*, **1**, 1297.
- BØYUM, A. (1976) Isolation of lymphocytes, granulocytes and macrophages. *Scandinavian Journal of Immunology*, **5**, Suppl. 5.
- CARAPETO, F.J., WINKELMANN, R.K. & JORDON, R.E. (1976) T and B Lymphocytes in contact and atopic dermatitis. *Archives of Dermatology*, **112**, 1095.
- COHEN, H.I. (1976) The immunology of atopic dermatitis, 1976. *Cutis*, **17**, 1186.
- CORMANE, R.H., HUSZ, S. & HAMERLINCK, F. (1974) Immunoglobulin- and complement-bearing lymphocytes in allergic contact dermatitis and atopic dermatitis (eczema). *British Journal of Dermatology*, **90**, 597.
- FRØLAND, S.S. (1972) Binding of sheep erythrocytes to human lymphocytes. A probable marker for T lymphocytes. *Scandinavian Journal of Immunology*, **1**, 269.
- FRØLAND, S.S. & NATVIG, J.B. (1972a) Class, subclass, and allelic exclusion of membrane bound Ig of human lymphocytes. *Journal of Experimental Medicine*, **136**, 409.
- FRØLAND, S.S. & NATVIG, J.B. (1972b) Surface-bound immunoglobulin on lymphocytes from normal and immunodeficient humans. *Scandinavian Journal of Immunology*, **1**, 1.
- FRØLAND, S.S. & NATVIG, J.B. (1973) Identifications of three different human lymphocyte populations by surface markers. *Transplantation Reviews*, **16**, 114.
- GONZALES-MOLINA, A. & SPIEGELBERG, H.L. (1977) A subpopulation of normal human peripheral B lymphocytes that bind IgE. *Journal of Clinical Investigation*, **59**, 616.
- GROVE, D.I., REID, J.G. & FORBES, I.J. (1975) Humoral and cellular immunity in atopic eczema. *British Journal of Dermatology*, **92**, 611.
- HANIFIN, J.M. (1974) Immunological aspects of atopic dermatitis. *Cutis*, **13**, 479.
- HOVMARK, A. (1975) An *in vivo* and *in vitro* study of cell-mediated immunity in atopic dermatitis. *Acta dermato-venereologica (Stockholm)*, **55**, 181.
- HOVMARK, A. (1977) An *in vitro* study of depressed cell-mediated immunity and of T and B lymphocytes in atopic dermatitis. *Acta dermato-venereologica (Stockholm)*, **57**, 237.
- KING, M., ALAYE, N. & AUGUSTIN, R. (1976) Demonstration of reaginic antibodies on human basophils by immune adherence to allergen-coated Sepharose beads. *Clinical Allergy*, **6**, 339.
- LOBITZ, W.C., HONEYMAN, J.F. & WINKLER, N.W. (1972) Suppressed cell-mediated immunity in two adults with atopic dermatitis. *British Journal of Dermatology*, **86**, 317.
- LUCKASEN, J.R., SABAD, A., GOLTZ, R.W. & KERSEY, J.H. (1974) T and B lymphocytes in atopic eczema. *Archives of Dermatology*, **110**, 375.
- MAIZE, J. C. (1976) Atopic dermatitis. *International Journal of Dermatology*, **15**, 555.
- MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.
- MCGEADY, S.J. & BUCKLEY, R.H. (1975) Depression of cell-mediated immunity in atopic eczema. *Journal of Allergology and Clinical Immunology*, **56**, 393.
- OGAWA, M., BERGER, P.A., MCINTYRE, O.R., CLENDENNING, W.E. & ISHIZAKA, K. (1971) IgE in atopic dermatitis. *Archives of Dermatology*, **103**, 575.
- RAJKA, G. (1968) Delayed dermal and epidermal reactivity in atopic dermatitis. *Acta dermato-venereologica (Stockholm)*, **48**, 186.
- ROGGE, J.L. & HANIFIN, J.M. (1976) Immunodeficiencies in severe atopic dermatitis. *Archives of Dermatology*, **112**, 1391.
- STRANNEGÅRD, I.L., LINDHOLM, L. & STRANNEGÅRD, Ø. (1976) T lymphocytes in atopic children. *International Archives of Allergy and Applied Immunology*, **50**, 684.
- THESTRUP-PEDERSEN, K., ELLEGAARD, J., THULIN, H. & ZACHARIAE, H. (1977) PPD and mitogen responsiveness of lymphocytes from patients with atopic dermatitis. *Clinical and Experimental Immunology*, **27**, 118.
- WATANABE, N., KOJIMA, S. & OVARY, Z. (1976) Suppression of IgE antibody production in SJL mice. I. Non-specific suppressor T cells. *Journal of Experimental Medicine*, **143**, 833.
- ØHMAN, S. & JOHANSSON, S.G. (1974) Immunoglobulins in atopic dermatitis. With special reference to IgE. *Acta dermato-venereologica (Stockholm)*, **54**, 93.

Comparative electron microscopic study between Mendes da Costa's disease and recessive epidermolysis bullosa dystrophica

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SUMMARY

An electron microscopic study of Mendes da Costa's disease (MCD) has been undertaken to determine whether this disease is a variant of epidermolysis bullosa dystrophica, or should be classified as a separate disease entity. It was shown that in MCD lacunae occur in the lower layers of the epidermis, in the presence of normal anchoring fibrils. In the recessive form of epidermolysis bullosa dystrophica (EBD), however, no lacunae were found in the epidermis, and anchoring fibrils were absent. These findings support the concept that MCD should not be considered as a special form of epidermolysis bullosa dystrophica.

Under the denomination 'Typus maculatus der bullösen hereditären Dystrophie' Mendes da Costa and van der Valk described a distinctive disease in 1908. It affects only males and, besides the appearance of blisters, is characterized by the presence of more or less pronounced reticular erythema on the limbs, atrichia and hyper- and depigmentation. Microcephaly, dwarfism and alterations of the fingers and the nails have also been observed. The authors considered the disease as a special form of 'Epidermolysis bullosa hereditaria', although they noted that in contrast to what can be seen in epidermolysis bullosa, the blisters could not be provoked by trauma.

In a detailed study of the disorder, Carol & Kooy (1937) indicated obvious differences from epidermolysis bullosa dystrophica. The differences between MCD and EBD are clinical, histological and genetic (see Table 1). The clinical differences are:

The bullae with MCD cannot be provoked by trauma or artificial rubbing. In MCD the bullae only occur in early childhood whereas in EBD the symptoms often become manifest at a later age.

In MCD the mucosae have thus far never been affected. Furthermore, the atrichia and the remarkable reticular pigmentation have never been observed in EBD.

The histology of MCD reveals suprabasal bullae and a remarkable appearance of the basal cells; in EBD the bullae are always sub-basal.

TABLE 1. Clinical differences between Mendes da Costa's disease (MCD) and epidermolysis bullosa dystrophica (EBD)

	MCD	EBD
Trauma	No effect	Pos. effect
Artificial rubbing	No effect	Pos. effect
Atrichia	+	—
De- + hyperpigmentation	+	—
Nail deformities	+	±
Ocular manifestations (corneal maculae)	+	—
Mucosal involvement	—	+
Histological		
Bullae	Suprabasal	Sub-basal
Genetic	Sex-linked	Not sex-linked

In MCD there is a clear recessive sex-linked inheritance; EBD is not sex-linked.

Based on their observations, Carol & Kooy were therefore of the opinion that the disease described by Mendes da Costa and van der Valk is to be considered as a separate entity. These differences were also described by Woerdeman (1958), who pointed out that Touraine (1942) classified MCD as a variant of the third group (epidermolysse bulleuse polydysplasique) of EBD, likewise as epidermolysis bullosa letalis of Herlitz (1935).

MATERIALS AND METHODS

One skin biopsy was taken from a 17-year-old male with MCD and from a 21-year-old male with EBD. In the case with MCD the biopsy was taken from a blister and the adjuvant skin of the extensor aspect

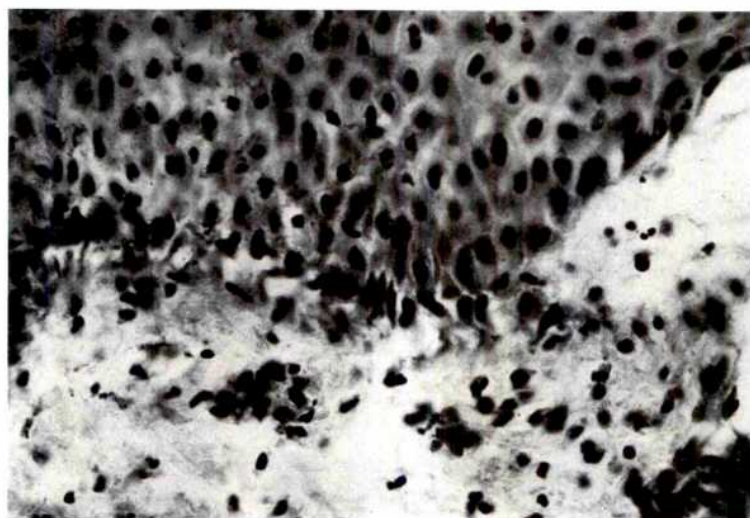


FIGURE 1. Mendes da Costa's disease. Histology. The cytoplasm of the basal cells adjacent to the blister is lacunar and the blister itself is covered by the cell layers located above the basal cells (H & E, $\times 385$).

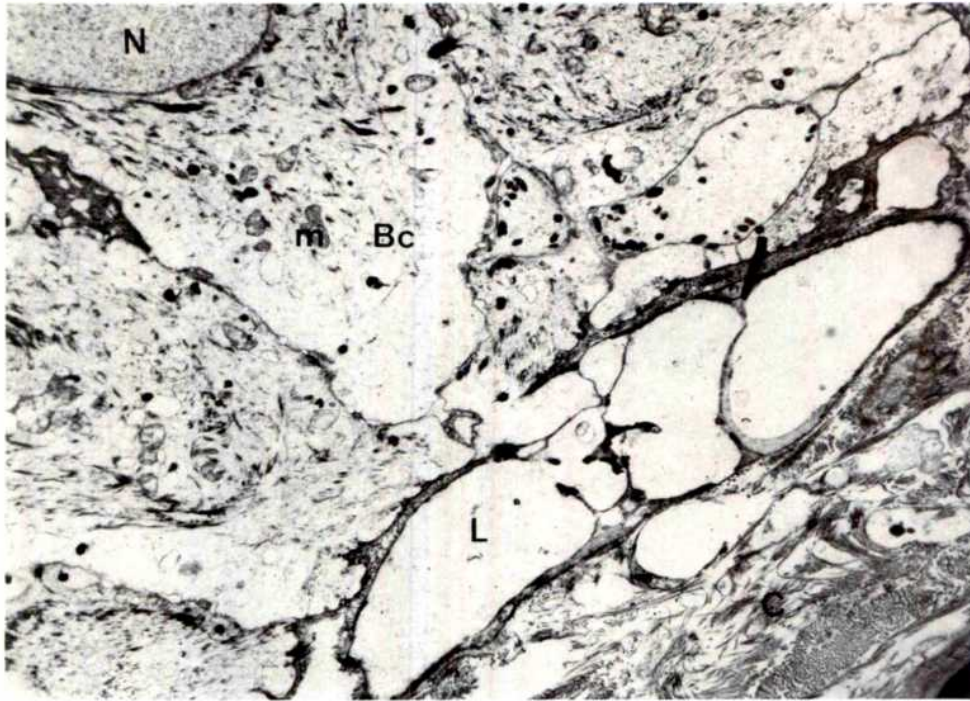


FIGURE 2. Mendes da Costa's disease. The cytoplasm of the basal cells is oedematous, and the cell membrane, as well as the basement membrane, is disrupted. Bc, basal cell; C, collagen fibrils; L, lacuna in the upper cutis; m, mitochondria; N, nucleus; t, tonofilaments ($\times 6000$).

of the thigh; in the patient with EBD a blister from the dorsum of the right hand and the adjacent skin was studied. For histological examination the biopsies were fixed in neutral formalin and treated in the classical way. For electron microscopy biopsy specimens were fixed in 2.5% glutaraldehyde buffered with 0.1 M Sørensen's buffer and postfixed in buffered 1% OsO_4 . The tissues were embedded in Durcupan ACM. Ultrathin sections were cut with a Reichert ultramicrotome and double stained with lead citrate and uranyl acetate (Reynolds, 1963). They were examined with a Siemens Elmiskop I electron microscope at 80 kV.

RESULTS

Histology

Mendes da Costa's disease. The cytoplasm of the cells of the epidermal basal cell layer adjacent to the blister is oedematous and lacunar (Fig. 1). The Malpighian layer is slightly spongiotic. The granular and the horny layers are normal. The blister, resulting from the changes in the basal cells, is covered by the cell layers of the epidermis located thereon. In the blister itself a few lymphocytes and polymorphonuclear cells can be seen. The bottom of the blister is composed of the papillary layer, upon which remnants of basal cells still adhere.

In the superficial cutis, lymphocytes and histiocytes are found surrounding the capillaries.

Recessive form of epidermolysis bullosa dystrophica. The skin surrounding the blister is normal. The

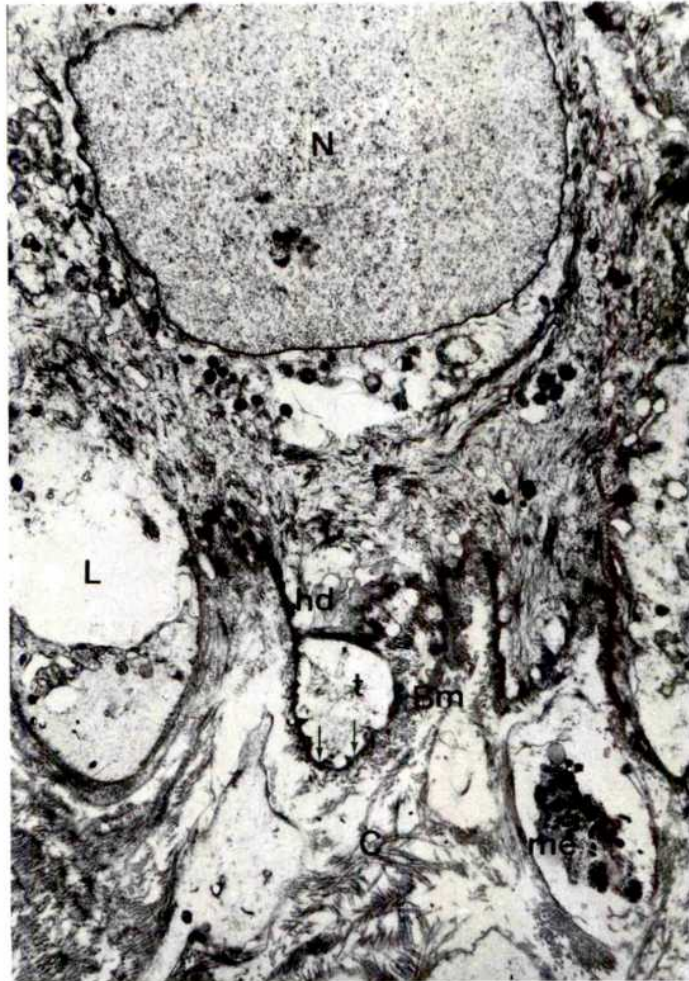


FIGURE 3. Mendes da Costa's disease. Lacunae are formed in the basal epidermal cells and the undermost cellular membrane is absent in several places. Melanosomes can be seen around the nucleus. Bm, basement membrane; C, collagen; hd, half desmosomes; L, intracytoplasmic lacunae in the basal cells; me, melanosomes; N, nucleus; t, tonofilaments; ↓, absence of the cellular membrane of the basal cell ($\times 7900$).

blister itself is empty and covered by the whole epidermis and its bottom is composed of the papillary layer. There is no infiltrate in the cutis.

Electron microscopy

To investigate blister formation in MCD as well as in EBD, the skin adjacent to a blister was studied.

Mendes da Costa's disease. Epidermis: The basal cells are cuboidal and surrounded by a smooth membrane which, at the lateral and upper sides of the cells, has very few desmosomes; the half-desmosomes on the undermost cell-membrane are normal. Between the half-desmosomes, the plasma membrane is often lacking or disrupted. The cytoplasm is oedematous and lacunar (Fig. 2); it contains a variable amount of mitochondria and a few thin and short tonofilaments scattered throughout



FIGURE 4. Mendes da Costa's disease. The cell membrane of the basal cell is disrupted. Under the basement membrane numerous anchoring fibrils can be observed. af, anchoring fibrils; Bm, basement membrane; hd, half desmosomes; ↓, rupture of the basal cell membrane; C, collagen ($\times 27,000$).

the cell. The nucleus is regular and surrounded by a normal double-layered membrane. The nucleolus is dense. In the perinuclear area melanosomes can be seen (Fig. 3). In the stratum mucosum the cells become diamond-shaped and the intercellular spaces grow wider. The Malpighian cells have numerous desmosomes; thin and short tonofibrils are scattered throughout the cytoplasm. The stratum granulosum and stratum corneum are morphologically normal.

Cutis: The dermal basement-membrane is 300–900 Å thick and is always present. When the cell membrane of the epidermal basal cells is unimpaired, the 'ad-epidermal' space between the basement membrane and the epidermal cells is of normal thickness (± 300 Å) and contains fine fibrils connecting both membranes. The anchoring fibrils are numerous in the upper cutis (Fig. 4). They measure 150–450 Å in width and are composed of non periodical dense and light bands. The cross-striated fibres of the papillary layer measure 250–700 Å and have a periodicity of 300–600 Å. In the deeper layers of the cutis the collagen is morphologically normal. The capillaries show no abnormalities.

Recessive form of epidermolysis bullosa dystrophica

Epidermis: the basal cells and the Malpighian cells show no pathological changes.

Cutis: the dermal basement membrane is not very conspicuous (Fig. 5). Consequently the thickness of the ad-epidermal space is difficult to judge. The anchoring fibrils are almost completely lacking. Blister formation could be demonstrated to occur underneath the basement membrane.

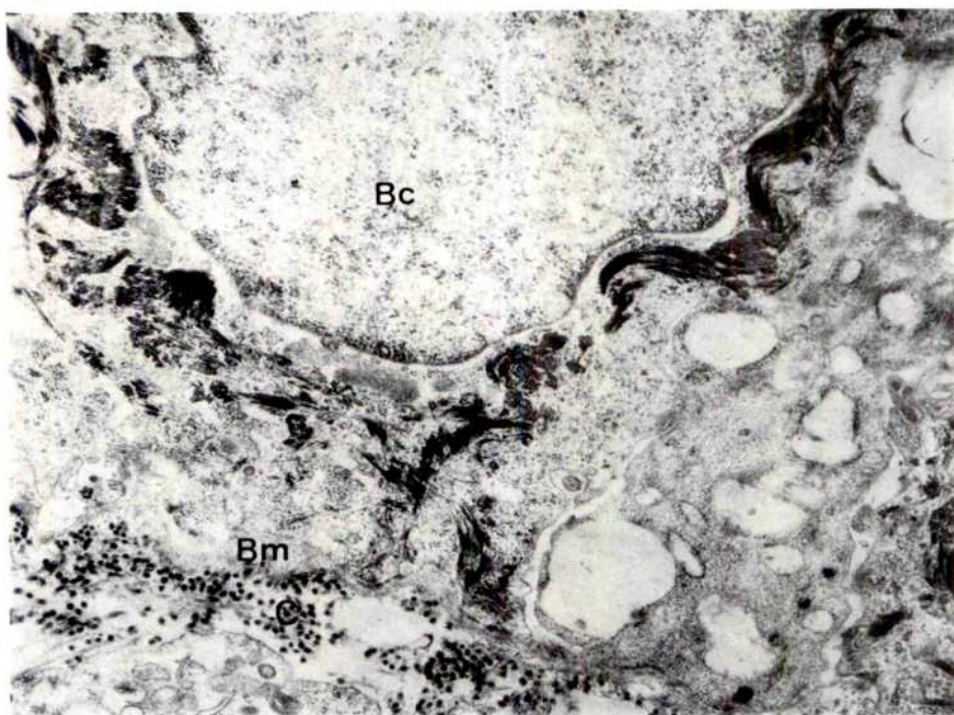


FIGURE 5. Recessive form of the epidermolysis bullosa dystrophica. No anchoring fibrils can be found under the basement membrane. Bc, basal cell; Bm, basement membrane (absence of anchoring fibrils); C, collagen ($\times 20,000$).

DISCUSSION

It appears from our study that under the light as well as under the electron microscope the most striking alterations in MCD are in the epidermal basal cells. In these cells the cytoplasm is lacunar and contains vacuoles which are the starting point of blister formation. Moreover, electron microscopic studies show that the basement membrane and anchoring fibrils are normal. There are no alterations of the collagen fibres. These findings are very different from the changes observed by us in recessive EBD, which shows the absence of anchoring fibrils and the blister underneath the basement membrane. The latter findings are in accordance with those of Pearson (1962), Ritzenfeld (1966), Vogel & Schnyder (1965), Briggaman & Wheeler (1973, 1975), Hashimoto *et al.* (1976a).

Later on, however, Vogel & Schnyder (1967), as well as Kobayashi (1967) and Neering & Wesseldijk (1974), reported that bulla formation in recessive EBD was located between the dermal basement membrane and the epidermal cell membrane. According to Neering & Wesseldijk (1974) blisters were assumed to result from the absence of half-desmosomes. However, Pearson (1962) suggested that the subepidermal blister formation should be ascribed to degenerative changes of collagen.

Subepidermal blister formation was found not only in recessive EBD but also in the dominant (Anton-Lamprecht & Schnyder, 1973) and acquired variants (Woerdeman, 1974; Gibbs & Minus, 1975; Metz, Frank & Metz, 1975). In epidermolysis bullosa simplex, however, changes in the basal cells occur in the presence of an intact basal membrane and in the absence of changes in the papillary layer (Pearson, 1962). In addition, Lapière, Castermans-Elias & Firket (1964) described vacuolization

of the cytoplasm of the epidermal basal cells in a patient with epidermolysis bullosa letalis Herlitz. In this disease, however, Pearson (1962, 1971), Pearson, Potter & Strauss (1974), Hashimoto, Anton-Lamprecht & Meyburg (1975) and Hashimoto *et al.* (1976b) observed the primary changes of blister formation in the 'ad-epidermal' or 'intermembrane' space separating the plasma membrane of the basal cells from the dermal basement membrane; Pearson (1961) and Hashimoto *et al.* (1976b) were of the opinion that the changes in the basal cells are a secondary phenomenon.

It can be concluded that primary changes in the basal cells were never described in the recessive form of epidermolysis bullosa dystrophica and can only be seen in the epidermolysis bullosa simplex (Pearson, 1962; Schnyder *et al.*, 1964). The intra-epidermal localization of the blister might explain the absence of scarring in this disease. As the clinical symptoms and the electron microscopic alterations of MCD are distinct from those of recessive EBD and EBD letalis, there is no indication to consider them related; the symptomatology of EBD letalis is furthermore so different from that of the MCD that it is not possible to consider them identical. These findings confirm the opinion of Carol & Kooy (1937) that MCD has to be considered a separate disease entity.

REFERENCES

- ANTON-LAMPRECHT, L. & SCHNYDER, U. (1973) Epidermolysis bullosa dystrophica dominans—ein Defekt der anchoring Fibrils? *Dermatologica*, **147**, 289.
- BRIGGAMAN, R.A. & WHEELER, C.E. (1973) Studies on the pathogenesis of recessive epidermolysis bullosa dystrophica. *Journal of Investigative Dermatology*, **60**, 109.
- BRIGGAMAN, R.A. & WHEELER, C.E. (1975) Epidermolysis bullosa dystrophica-recessive: a possible role of anchoring fibrils in the pathogenesis. *Journal of Investigative Dermatology*, **65**, 203.
- CAROL, W.L.L. & KOOY, R. (1937) Typus maculatus der bullösen hereditären Dystrophie. *Acta dermato-venereologica*, **18**, 265.
- GIBBS, R.B. & MINUS, H.R. (1975) Epidermolysis bullosa acquisita with electron microscopical studies. *Archives of Dermatology*, **111**, 215.
- HASHIMOTO, I., ANTON-LAMPRECHT, I. & MEYBURG, P. (1975) Epidermolysis bullosa hereditaria letalis: Report of a case and probable ultrastructural defects. *Helvetica paediatrica Acta*, **30**, 543.
- HASHIMOTO, I., SCHNYDER, U.W., ANTON-LAMPRECHT, I., GEDDE-DAHL, T. Jr & WARD, S. (1976a) Ultrastructural studies in epidermolysis bullosa hereditaria. III. Recessive dystrophic types with dermolytic blistering (Hallopeau-Siemens types and inverse type). *Archives for Dermatological Research*, **256**, 137.
- HASHIMOTO, I., GEDDE-DAHL, T., SCHNYDER, U.W. & ANTON-LAMPRECHT, I. (1976b) Ultrastructural studies in epidermolysis bullosa hereditaria. IV. Recessive dystrophic types with junctional blistering (infantile or Herlitz-Pearson type and adult type). *Archives for Dermatological Research*, **257**, 17.
- HERLITZ (1935) Kongenitaler nicht syphilitischer Pemphigus. Ein Übersicht nebst Beschreibung einer neuen Krankheitsform (epidermolysis bullosa hereditaria lethalis). *Acta Paediatrica*, **17**, 315.
- KOBAYASI, T. (1967) Dermo-epidermal junction in the recessive type of epidermolysis bullosa. An electron-microscope study. *Acta dermato-venereologica*, **47**, 57.
- LAPIERE, S., CASTERMANS-ELIAS, S. & FIRKET, H. (1964) Elektronenmikroskopische Untersuchungen über die Ultrastruktur der Epidermolysis bullosa letalis bei einem Säugling mit familiärer Belastung. *Hautarzt*, **15**, 30.
- MENDES DA COSTA, S. & VAN DER VALK, J.W. (1908) Typus maculatus der bullösen hereditären Dystrophie. *Archiv für Dermatologie und Syphiligraphie*, **19**, 3.
- MENDES DA COSTA, S. & VAN DER VALK, J.W. (1908) Dystrophia bullosa hereditaria, typus maculatus. *Nederlands Tijdschrift voor Geneeskunde*, **52**, 660.
- METZ, J., FRANK, H. & METZ, G. (1975) Zur Pathomorphogenese der Blasenbildung bei Epidermolysis bullosa acquisita und Epidermolysis bullosa dystrophica. *Archiv für Dermatologische Forschung*, **254**, 103.
- NEERING, H. & WESSELDIJK, W. (1974) Recessive epidermolysis bullosa et albopapuloidea without nail dystrophy and scarring. *Dermatologica*, **149**, 180.
- PEARSON, R.W. (1962) Studies on the pathogenesis of epidermolysis bullosa. *Journal of Investigative Dermatology*, **39**, 551.
- PEARSON, R.W. (1971) The mechanobullous diseases. In: *Dermatology in General Medicine* (Ed. by T.B. Fitzpatrick,

- K.A.Arndt, W.H.Clark, Jr, A.Z.Eisen, E.J.Van Scott and J.H.Vaught), pp. 621-643, McGraw Hill, New York.
- PEARSON, R.W., POTTER, B. & STRAUSS, F. (1974) Epidermolysis bullosa hereditaria letalis. Clinical and histological manifestations and course of the disease. *Archives of Dermatology*, **109**, 349.
- REYNOLDS, E.S. (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology*, **17**, 208.
- RITZENFELD, P. (1966) Zur Histogenese und Differentialdiagnose hereditären Epidermolysen. *Archiv für klinische und experimentelle Dermatologie*, **224**, 128.
- SCHNYDER, U.W., JUNG, E.G. & SALAMON, T. (1964) Zur klassifizierung Histogenetik, Gerinnungsphysiologie und Therapie der hereditären Epidermolysen. *Archiv für klinische und experimentelle Dermatologie*, **220**, 38.
- TOURAINE, M.A. (1942) Classification des épidermolyses bulleuses. *Annales de Dermatologie et Syphiligraphie*, 8ème Série, **2**, 309.
- VOGEL, A. & SCHNYDER, U.W. (1965) Elektronenmikroskopische Untersuchungen an der rezessiven Epidermolysis bullosa dystrophica. *Dermatologica*, **131**, 81.
- VOGEL, A. & SCHNYDER, U.W. (1967) Feinstrukturelle Untersuchungen an rezessiv-dystrophischer Epidermolysis bullosa hereditaria. *Dermatologica*, **135**, 149.
- WOERDEMAN, M.J. (1958) Dystrophia bullosa hereditaria, typus maculatus. *Nederlands Tijdschrift voor Geneeskunde*, **102**, 111.
- WOERDEMAN, M.J. (1974) Epidermolysis bullosa dystrophica acquisita. *Dermatologica*, **149**, 184.

Skin temperature changes induced by UV irradiation at 254 nm and 300 nm

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SUMMARY

Skin temperatures 24 h after irradiation at both 254 and 300 nm have been examined using the technique of thermography. Increases in skin temperature have been recorded at both wavelengths but the magnitude of these differences after 254 nm irradiation is influenced by the ambient temperature. The apparent disagreement between two groups of observers working in the field of UV erythema may well have been explained.

Although UV therapy has been used for several decades for various skin conditions, until recently very little work has been carried out investigating blood flow changes induced by irradiation. One method which has been used to study cutaneous blood flow changes is skin temperature, where an increase in temperature is considered to reflect an increase in arterial inflow. An early investigator in this field was van der Leun (1966) who found an increase in skin temperature after irradiation at 300 nm but not at 254 nm, although at this wavelength he gave doses up to 30 times that required to cause a just perceptible erythema (MED). On the other hand, Ramsay & Cripps (1970) using a photoelectric plethysmograph showed increased pulsation in skin irradiated at both 254 nm and 300 nm which they interpreted to mean an increase in blood flow. However, this conclusion has been criticized by van der Leun (1972). Further studies by Ramsay & Challoner (1976) have been more exhaustive and they measured skin blood flow by two separate methods (photoelectric plethysmography and thermal clearance) as well as skin temperature. In these studies, there was again an increase in both pulsations and skin temperature at 254 and 300 nm.

We were worried by this total disagreement in the skin temperature changes after irradiation with UV at 254 nm and decided to look further into this problem. One fundamental difference between the experiments of van der Leun and of Ramsay and his co-workers was in the method of measuring skin temperature. Ramsay used surface contact devices (thermometers) whereas van der Leun thought that the contact may upset the temperature patterns and he used a device which consisted of an arsenic

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trisulphide lens on a vacuum thermopile and measured the infra-red emission (dependent on skin temperature) of the skin.

We felt that it may be the measurement technique which had led to these two opposing opinions and we decided to measure the skin temperature changes caused by irradiation at both 254 nm and 300 nm using a method essentially the same as that used by van der Leun. The technique used was that of thermography, a system which images the infra-red (2.0–5.6 μm) emission of the skin analogous to a thermal television picture and allows accurate measurement of skin temperature.

MATERIALS AND METHODS

A total of 13 volunteer subjects were tested, ten males and three females. The age range was 23–65 years and all subjects were untanned and showed no clinical sign of any skin abnormalities on the back (the area studied in these experiments). The UV source for irradiation at 254 nm was a Philips TUV germicidal lamp which has a nearly monochromatic output and at 300 nm a Westinghouse 'Sunlamp' which has a continuum which peaks very near 300 nm. These lamps are directly comparable to those sources used by van der Leun (1966) and the same lamps used by Ramsay & Challoner (1976). All subjects were irradiated at 254 nm on three areas with doses of 140, 370 and 520 mJ.cm^{-2} respectively. Only 5 subjects were irradiated at both 254 and 300 nm; the three 254 nm areas were on the right side of the back and the three 300 nm on the central lateral position on the left side. The doses at 300 nm were 220, 440 and 660 mJ.cm^{-2} . The doses at both wavelengths were sufficient to produce an erythema on all subjects, mild for the lower doses and quite marked at the highest. The lamps used in these experiments have been tested and a typical MED at 254 nm is 35 mJ.cm^{-2} and at 300 nm 200 mJ.cm^{-2} . The latter figure is greater than that reported for monochromatic radiation but has been explained by Challoner (1973).

It was felt that 5 subjects at 300 nm would be sufficient to verify the experimental technique since there has never been any disagreement between the observed temperature changes after irradiation with this wavelength by any observer.

All skin temperature measurements were made at 24 ± 1 h after irradiation. An AGA 680 medical Thermovision, thermographic camera was used to determine skin temperature. This camera measures the quantity of infra-red radiation being emitted by the skin and displays the result as a television type picture in either monochrome or colour. Since it allows accurate quantification, the colour presentation, known as a colour isothermogram, was used to record the results presented in this paper.

Irrespective of pigmentation, clean, healthy skin, behaves like an ideal thermal solid in the infra-red region to which our AGA thermovision camera responds (2.0–5.6 μm) and the quantity of energy emitted in this region is therefore directly proportional to the fourth power of the absolute temperature. Furthermore it is possible to calibrate the display directly in terms of temperature by using a 'black body' temperature reference source.

An important feature of thermographic cameras is that they produce an almost instantaneous image, so that in our experiments we have been able to monitor skin temperature continuously and record any changes as they occurred.

During these experiments early measurements of the 254 nm areas were made at a room temperature of 22°C with the patient sitting stripped to the waist for about 15 mins to allow him to equilibrate before any recordings were taken. Although in all cases a temperature increase was observed, this was often rather low, being in most cases less than 1°C greater than unirradiated skin for the 560 mJ.cm^{-2} areas and comparably less for the others. The experience of one of the authors with skin temperature measurements (Challoner, 1973) suggested that these differences should be greater. In the work of Ramsay & Challoner (1976) skin temperatures were measured with the room temperature in the range

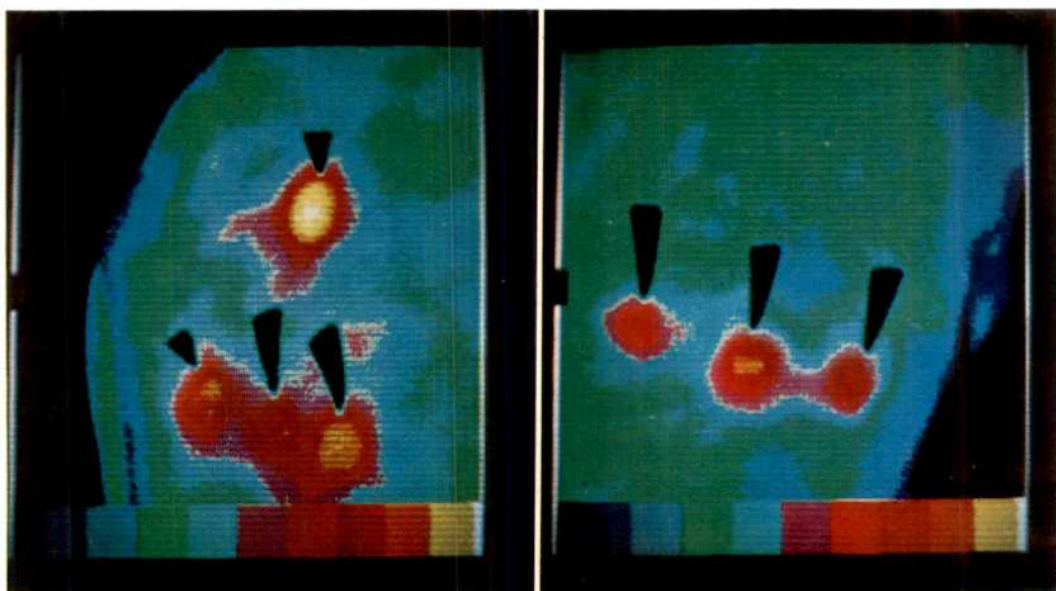


PLATE 1. Thermogram of a back taken at room temperature of 25°C . Four areas on left of figure were irradiated at 300 nm, doses given were $220 \text{ mJ}\cdot\text{cm}^{-2}$ for bottom left and centre area, $440 \text{ mJ}\cdot\text{cm}^{-2}$ for bottom right and $660 \text{ mJ}\cdot\text{cm}^{-2}$ for top centre. The three areas on the right of the figure were irradiated at 254 nm with doses of 140, 370, $520 \text{ mJ}\cdot\text{cm}^{-2}$ from left to right. Each colour represents $^{\circ}\text{C}$ temperature change and the mid-colour (mauve) represents 32.5°C .

23.5–25°C and we considered that this may be why we were not getting such noticeable differences. Accordingly, for one subject thermograms were taken with the room temperature initially at 22°C and sequentially as the room was heated up to 25°C. It was observed that as the room heated up the temperature differences due to irradiation at both 254 nm and 300 nm increased and, in fact, areas which could not be detected thermographically at 22°C were clearly observable at 25°C. Later thermograms for the subjects who had been irradiated at both wavelengths were taken with room temperature in the range 24–25°C.

RESULTS

In all subjects there was an increase in skin temperature after irradiation at both wavelengths. This was observable for all areas both at 254 and 300 nm when measured at the higher room temperature; the differences in the highest dose areas being in some cases up to 3°C (Plate 1). However, when tests were made at room temperature of 22°C, only the highest doses showed a clear increase at 254 nm but the lower doses in some cases were not recordable.

DISCUSSION

The purpose of this study was to investigate the cause of the conflicting published data on the temperature of skin, irradiated with UV light having a wavelength of 254 nm. At higher ambient temperatures, the observed increase in skin temperatures induced by the irradiation were in good agreement with those obtained by Ramsay & Challoner (1976). However at lower ambient temperatures, it was difficult to show an increase in skin temperature caused by the UV radiation. It is known that the skin is involved in the thermoregulation process and when sitting unclothed in a cool environment the skin blood flow drops to reduce the thermal loss through the skin. Furthermore, a decrease in skin temperature is associated with this decrease in blood flow. It is possible that this effect is much greater than that induced by the UV radiation and hence it appears to mask the changes caused by the UV irradiation.

Van der Leun performed all of his measurements on the volar surface of the forearm in a room with a constant temperature of 21°C. His choice of this temperature was because he felt that this was a best compromise. Lower room temperature, he stated, 'may lead to the arm becoming cold and suppressing any temperature differences, whilst higher room temperatures caused the veins to protrude from the arm and disturb the temperature pattern'. We chose the back for the experimental sites in order to minimize the disturbing influence of subcutaneous vessels; indeed we have found that the best thermograms, such as those shown in the figure, are obtained when the skin is insulated from the deeper vessels by a thick layer of subcutaneous fat.

The fact that van der Leun was able to measure increase in skin temperature after 300 nm irradiation, even with the colder room temperature, may be explained by the fact that 'MED for MED', 300 nm is more reactive than is MED 254 nm (see Challoner, 1973). With an ambient temperature of 21°C we have been able to obtain clear increases in skin temperature even at comparatively low doses of 300 nm radiation; furthermore, most workers agree that increasing doses at 300 nm produces progressive 'deepening' of the erythema, a phenomenon which is not so clearly observed with 254 nm radiation. Recent experiments have shown (Michell *et al.*, 1977) that, using the skin blood pulse as a criterion, 254 nm reaches a plateau phase after relatively low doses, whereas at 300 nm there is a much greater and progressive increase in pulsations until this plateau is reached.

The results obtained in this study show clearly that at ambient temperatures greater than 25°C there is a measurable increase in skin temperature after irradiation with both 254 and 300 nm UV. We believe that the reason that van der Leun was not able to measure an increase in skin temperature at 254 nm was most probably due to the ambient temperature, at which experiments were performed, being below this critical temperature.

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REFERENCES

- CHALLONER, A.V.J. (1973) Isotopic, optical and thermal conduction studies of skin vascularity changes induced by X-rays and ultraviolet light. Ph.D. Thesis, Polytechnic of Central London.
- MICHELL, P., MAGNUS, I.A. & CHALLONER, A.V.J. (1977) *To be published.*
- RAMSAY, C.A. & CHALLONER, A.V.J. (1976) Vascular changes in human skin after ultraviolet radiation. *British Journal of Dermatology*, **94**, 487.
- RAMSAY, C.A. & CRIPPS, D.J. (1970) Cutaneous arteriolar dilatation elicited by ultraviolet radiation. *Journal of Investigative Dermatology*, **54**, 332.
- VAN DER LEUN, J.C. (1966) Ultraviolet erythema. A study on diffusion processes in human skin. Ph.D. Thesis, Utrecht.
- VAN DER LEUN, J.C. (1972) On the action spectrum of ultraviolet erythema. In: *Research Progress in Organic, Biological and Medicinal Chemistry* (Ed. by U.Gallo and L.Santa Maria), Part II, vol. 3, p.711. North-Holland Publishing Co., Amsterdam.

Skin problems associated with multifunctional acrylic monomers in ultraviolet curing inks

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SUMMARY

Fifteen cases of contact dermatitis and three of irritant contact conjunctivitis due to multifunctional acrylic monomers in printing inks are described. Seven with dermatitis were sensitized to pentaerythritol triacrylate but evidenced no cross-reactivity to methyl methacrylate. The other eight cases of dermatitis were irritant. Pentaerythritol triacrylate has been shown experimentally to be a cutaneous sensitizer for guinea-pigs and man. Multi-functional acrylates should be handled with minimal skin contact, to avoid cutaneous problems.

Conventional inks and coatings are dried either by evaporation of a solvent used to dilute them, by absorption of the vehicle by the printed surface, or by oxidation (Dunn *et al.*, 1966). If applied to a metal surface, gas ovens are used to vaporize the solvent.

Environmental protection legislation in certain jurisdictions requires that gaseous solvent so produced be removed from plant effluents. This requires costly engineering to clear oven exhausts of solvent. Decreased gas supplies are making this method expensive. This has given impetus to a new solventless technology requiring less energy. It is based on three principal components (Shahidi, Trebellas & Vona, 1974; Rybny, 1974; Shahidi & Powanda, 1975).

Synthetic resins (i.e. plastics) with terminal acrylate radicals, such as acrylated epoxy resins, acrylated polyesters, acrylated alkyds and acrylated vegetable oils (e.g. acrylated linseed oil), are used (Fig. 1). A cross-linking acrylic monomer with more than one acrylate radical allows rapid chemical binding between the resinous polymers during curing. Three widely used multifunctional acrylic monomers (MFAs) are pentaerythritol triacrylate (PETA), trimethylolpropane triacrylate (TMPTA) and hexanediol diacrylate (HDODA) (Fig. 2).

Table 1 lists other acrylate cross-linking agents that are either less widely used, or are at present in the development stage. MFA's are of low viscosity and act as diluents. With curing, the diluent is bound in the finished material, resulting in no loss of coating thickness.

The MFAs may be compared with polyfunctional amine hardening agents used to cure epoxy resins. They differ in that no significant binding occurs until energy is supplied to the reaction from without. The energy source is ultraviolet light (UVL), with an intense continuous emission between

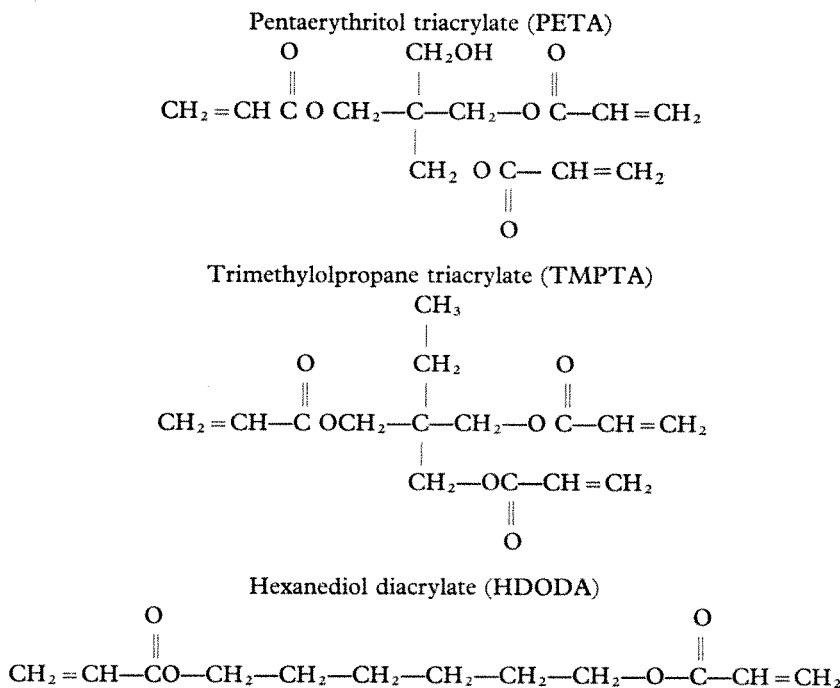


FIGURE 1. Three possible acrylated resins.

the wavelengths 300 and 400 nm. Into the polymer-monomer system a light absorbing agent is added, such as an aromatic ketone, benzoin or chlorinated aromatic compound. This chemical absorbs photons of UVL, then transfers the energy to the MFA by homolytic fragmentation of the photoinitiator, or excitation of the photoinitiator to a triplet state with electron transfer to the MFA. The activated MFA then binds to the acrylate ends of the polymers, effecting cure. This light induced hardening is almost instantaneous under optimum conditions.

Printing presses and coating machines are fitted with ultraviolet light encasements which expose the printed or coated surface to ultraviolet light (Fig. 3). Immediate drying results, allowing increased press speed, more complicated multicolour operations, elimination of smudging of printed surfaces and minimization of storage space for printed material.

Gas ovens can be eliminated for metal printing, effecting ecological and energy conservation. Application of this technical change is occurring rapidly for the above reasons (Rybny, Trebellas & Vona, 1975).

A new photo-engraving process has been devised which involves this technology. A negative is placed over a plate covered with a layer of light-sensitive acrylate. Following ultraviolet exposure, acrylate under the transparent areas of the negative hardens. The plate is then washed with solvent to remove the unreacted soluble acrylate, leaving the photo-engraved relief of the negative. The finished plate can be used directly in printing. Light-sensitive acrylate plates are known by the trade names Dycril and Nyloprint in Europe (Rycroft, 1977). Details of the chemical reactions involved in this process are not known, but the mechanism is probably similar to that I have outlined for ultraviolet curing inks.

This technology has been applied to dentistry, where a coating is applied to the teeth then cured with UVL. This is superior to past self-curing acrylate dental sealants, as no loss of mass occurs with drying, which led to incomplete sealing with earlier materials (Rock, 1977).

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FIGURE 2. The three multifunctional acrylate monomers presently in common use.

TABLE I. Less widely used multifunctional acrylic monomers

Diethylene glycol diacrylate
Tetraethylene glycol diacrylate
Trimethylolpropane trimethacrylate
Neopentaethylene glycol diacrylate
Dipentaerythritol mono hydroxy pentaacrylate

This report describes skin and conjunctival reactions arising in workers exposed to UV inks and their components. All worked in one ink manufacturing plant in Toronto, Canada, except one printer.

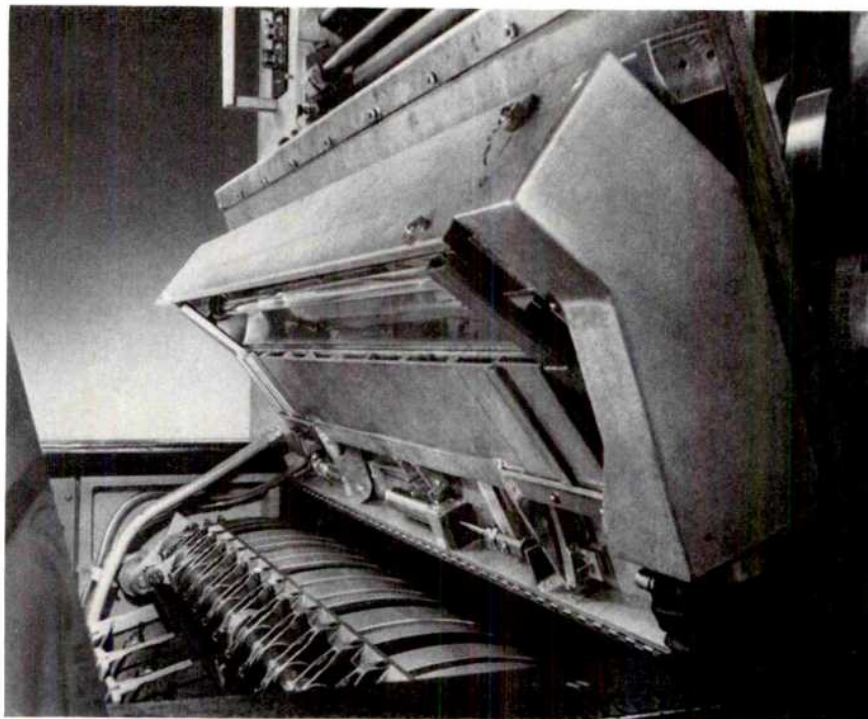


FIGURE 3. A printing press equipped with ultraviolet curing apparatus consisting of a fluorescent tube and reflector.

CLINICAL FEATURES

Ink manufacturing in the plant involved receiving raw materials, weighing and mixing them, grinding the mixture with three roller mills (Fig. 4), and packaging and shipping the finished product. These steps were carried out by receivers, mixers, packagers and shippers respectively.

Laboratory samples were regularly taken from finished inks for tests. Laboratory workers handled the finished inks and formulated test inks with raw materials.

Table 2 summarizes clinical findings in 19 patients. Case 19 operated an ultraviolet-curing printing press in a plant which fabricated printed metal cans. He was exposed only to finished ink.

Eleven patients had eye or skin problems but had no positive patch test reactions. They are thought to have had an irritant contact dermatitis (8 cases), or irritant contact conjunctivitis (3 cases). Two with dermatitis and one with conjunctivitis had problems when working near mills grinding UV ink, while having no direct contact with it, suggesting an airborne method of contact.

Seven cases of dermatitis had positive patch tests. One (Case 10) had skin problems when working near UV ink milling operations. The eruption involved his face, anterior neck and exposed areas of arms and hands, suggesting airborne contact.

CLINICAL INVESTIGATION

(a) Materials and methods

Patch tests were carried out with the chemicals as outlined in Tables 3 & 4. PETA, TMPTA and

TABLE 2. Clinical findings in workers with cutaneous or eye complaints

Case	Symptoms	Signs	Diagnosis	Job description
1.	Itching on exposed skin when mixing	Faint erythema of arms, face, neck & underside of the chin	Irritant contact dermatitis (airborne)	Mixer
2.	Eye irritation of 2-3 months duration aggravated by exposure to UV raw materials	Conjunctival hyperaemia. Firm erythematous 5 mm tumour on left lower eyelid	Irritant contact conjunctivitis, Chalazion	Foreman
3.	Itching of eyelids when working	Erythematous, scaling, indistinct patches on upper eyelids	Irritant contact dermatitis	Foreman
4.	'Burning' eyes when milling UV inks with high PETA concentration	Conjunctival hyperaemia	Irritant contact dermatitis (airborne)	Mill worker
5.	'Itchy' rash on wrists & hands when working with UV inks	Nil	Irritant contact dermatitis	Laboratory worker
6.	Itching, redness of face, hands & neck when cleaning some vats used for UV materials	Erythematous, scaling patches on the face, chin, neck & dorsa of hands	Irritant contact dermatitis (airborne)	Vat cleaner
7.	Itching of eyelids when working with UV inks	Erythematous, scaling, indistinct patches on upper eyelids	Irritant contact dermatitis	Mixer
8.	Itchy rash right upper eyelid	Erythematous, scaling, indistinct patch on the eyelid	Irritant contact dermatitis	Laboratory worker
9.	Itchy rash on arms and hands when working with UV inks	Erythematous, scaling, indistinct patches on arms & hands	Irritant contact dermatitis	Packaging finished inks
10.	Recurrent itchy rash on arms & hands later unable to pass by a mill processing UV ink without a later itchy rash on exposed skin	Erythematous, scaling, confluent patches on entire face, neck, arms, hands sparing clothed areas	Allergic contact dermatitis (airborne)	Mixer
11.	Itchy rash on arms & abdomen	Erythematous, scaling patches with indistinct borders on fore-arms, hands & abdomen	Allergic contact dermatitis	Mixer
12.	Itchy rash left cheek	Erythematous, scaling, indistinct patch left cheek	Allergic contact dermatitis	Mixer
13.	Itchy rash on the hands & eyelids	Erythematous, scaling, indistinct patches on eyelids & hands	Irritant contact dermatitis	Laboratory worker
14.	Itchy rash on left forearm	Erythematous, scaling, indistinct patch on left forearm	Allergic contact dermatitis	Shipper & receiver
15.	Itchy patches on thighs	Erythematous, scaling patches with indistinct borders under the trouser pockets	Allergic contact dermatitis	Shipper & receiver
16.	Itchy rash on hands	Erythematous, scaling, indistinct patches on dorsa of hands	Allergic contact dermatitis	Mill worker
17.	Severe burning & lacrymation of left eye after splashing UV ink into it	Conjunctival hyperaemia & increased lacrymation	Irritant contact conjunctivitis	Mill worker
18.	Swelling of the face, lips & hands for 6 months with aggravation when working with UV inks	Weals on torso & arms, dermatographism	Chronic urticaria	Mixer
19.	Itchy rash on backs of hands	Erythematous, scaling, indistinct patches on dorsa of hands	Allergic contact dermatitis	Printer

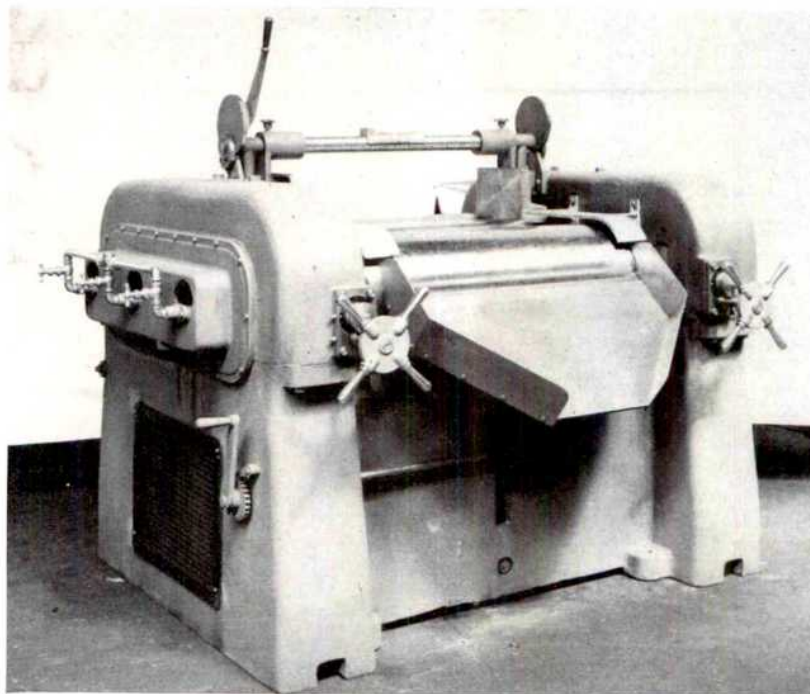


FIGURE 4. A three roller mill used to grind ink.

TABLE 3. Chemicals and their concentrations in petrolatum used to patch test Cases 1-7

Chemical	Concentration (%)
MMA	1
PETA	0.1
TMPTA	0.1
HDODA	0.1
Benzophenone	10
Dibutylphthalate	20

TABLE 4. Chemicals and their concentrations in petrolatum used to patch test Cases 8-15 and Case 19

Chemical	Concentration (%)
MMA	1
PETA	0.1
TMPTA	0.1
HDODA	0.1
Benzophenone	10
Dibutylphthalate	20
Linseed oil acrylate	1
Epoxy acrylate (a)	1
Epoxy acrylate (b)	1
Epoxy acrylate (c)	1
Thioxanthone	20
Diethoxyacetophenone	20
Linseed oil	As is
4,4' dimethylaminobenzophenone	50

HDODA concentrations used were determined not to be irritant, with 15 control subjects. The substances were applied to the upper back on 'Al-Test Strips' (Hollister-Stier Ltd.). The sites were examined after 48 h, with time allowed for the pressure effect of the 'Al-Test Strip' to resolve completely. Patients were instructed to call if further sites of skin reaction appeared after examination.

(b) *Results*

Table 5 outlines the responses of those who reacted to one or more of the chemicals. Two reacted to TMPTA and none to HDODA. None reacted to methyl methacrylate (MMA). Case 10 did not

TABLE 5. Cutaneous reactions in those patients with positive patch tests

Case	Chemical				
	HDODA	TMPTA	PETA	MMA	Cured UV ink
10	0	0	3+	0	0
11	0	2+	3+	0	NT
12	0	0	2+	0	NT
14	0	0	2+	0	NT
15	0	0	3+	0	NT
16	0	0	1+	0	NT
19	0	2+	3+	0	NT

0 = negative; 1+ = macular erythema; 2+ = oedematous or vesicular reaction; 3+ = spreading, bullous or ulcerative reaction.

react to the cured UV ink. Case 18 was not tested, as his disease appeared not to be occupationally related.

None of the patients tested with differing molecular weight epoxy acrylates and linseed oil acrylate evidenced reactivity.

EXPERIMENTAL INVESTIGATION

(a) *Material and method for the animal study*

Animals. Fifteen albino Hartley/Dalkin strain guinea-pigs weighing 300–500 g were obtained from the Camm Research Institute, Wayne, New Jersey, U.S.A. They were housed in separate cages. Purina Rabbit Chow, carrots, lettuce and fresh water were provided *ad libitum*.

Sensitization. The animals were exposed to PETA provided by the Celanese Chemical Company, New York, in two stages, as described by Magnusson & Kligman (1970). The first stage involved intradermal injections into the shoulder region after topical and intradermal irritancy tests had been performed on 20 control animals. A row of 3 injections of the following compositions were made on each side in the experimental animals:

- (1) 0.1 ml adjuvant (Freund's complete adjuvant, Difco Laboratories, Detroit, Michigan).
- (2) 0.1 ml of 0.05% PETA in propylene glycol.
- (3) 0.05 ml of 0.1% PETA in propylene glycol mixed with 0.05 ml of adjuvant.

Each animal received six 0.1 ml injections into the shoulder region.

One week after injection, the shoulder was clipped and shaved. The second part of the sensitization involved topical application. For 48 h the animals were exposed to PETA 25% in petrolatum, on a 2 × 4 cm 3MM Whatham filter paper. The patch was held in position by a layer of impervious plastic tape (3M Blenderm tape) covered by a 3 in. elastic adhesive bandage (Elastoplast, Smith & Nephew Ltd.) wrapped about the animal. All bandages remained in place throughout the 48 h period.

Challenge. All animals were challenged 2 weeks following topical exposure. A 5 × 5 cm area on the flank was clipped and then shaved. PETA 10% in petrolatum was applied to a 2 × 2 cm patch of

3MM Whatham filter paper. The patch was held in place in the same manner as for topical induction above, except that it was removed at 24 h. The sites were read 24 h after removal of the bandage, using the following grading system: 0 = no reaction; 1+ = scattered mild redness; 2+ = moderate and diffuse redness; 3+ = intense redness and swelling.

(b) *Results of the animal study*

Thirteen of the fifteen animals (87%) reacted positively to the monomer. Three of those reacting were graded greater than 1.

PETA at concentrations greater than 10% in petrolatum induced inflammation in most of the control animals.

(c) *Material and method for the human study*

Subjects. Eleven paid human volunteers gave informed consent to the application of PETA to their skin. Their ages ranged from 18 to 25.

Sensitization. PETA 10% in petrolatum was applied to the extensor surface of the arm on an 'AI-Test Strip' covered with Dermicel Tape. The patch was removed daily and the site was examined. The patch test was reapplied only if there was no evidence of inflammation.

Challenge. Nine of the eleven subjects were available for reassessment 4 weeks after the initial patch tests were applied. Two were lost to follow-up. One subject refused challenge tests as he had developed persistent eczematous dermatitis at the test site, which was still present at the time he was recalled.

Patch tests were carried out with PETA 0.1% in petrolatum on 'AI-Test Strip' applied to the upper back with 'Dermicel' tape. The patches were removed at 48 h and scored for reaction after

TABLE 6. Results of patch tests on experimental subjects

Subject	Reaction at 48 h
1	3+
3	0
4	2+
5	1+
6	NDS
7	2+
8	2+
9	2+
11	0

0 = negative reaction; 1+ = macular erythema; 2+ = oedematous or vesicular reaction; 3+ = spreading, bullous or ulcerative reaction; NDS = patch test refused due to active eczematous dermatitis.

pressure effects had resolved according to the following grading system: 0 = no reaction; 1+ = macular erythema; 2+ = oedematous or vesicular reaction; 3+ = spreading, bullous or ulcerative reaction.

Subjects were instructed to return if a reaction occurred at the site following this observation at 48 h.

(d) *Results of the human study*

Table 6 lists the results of the challenge tests. Seven of nine subjects available for reassessment reacted positively to 0.1% PETA in petrolatum. Using Log-Probit analysis as described by Kligman & Wooding (1967), PETA 10% in petrolatum has an irritancy time for 50% of the subjects (IT_{50}) of 0.94 days.

COMMENT

MMA is an established human contact allergen (Harris, 1953; Allardice, 1957; Magnusson & Mobacken, 1972; Magnusson & Mobacken, 1972; Pegum & Medhurst, 1971; Nyquist, Koch & Magnusson, 1972; Fisher, Franks & Glick, 1957; Crissey, 1965; Spiechowicz, 1971) and irritant (Routledge, 1971; Goodwin, 1976). Since 1880 it has been known to polymerize, and its use has been extensive since the late thirties (Caress, 1944). In the medical field alone it enjoys wide use in dentistry (Nyquist, Koch & Magnusson, 1972), veterinary medicine (Jenny, Evans & Raker, 1965), optometrics and orthopaedic surgery (Lavorgna *et al.*, 1972). In view of its large scale use and resultant exposure to skin for over 40 years, published reports of cutaneous disease related to it are few. It is considered an important cause of contact dermatitis by a number of experts (Adams, 1969; Fisher, 1973; Fregert, 1974; Fregert & Hjorth, 1972), suggesting that more cases are occurring than the number of reports would suggest. Spiechowicz reports an incidence of MMA sensitization in dental technicians of 10%, after 2–14 years of exposure (Spiechowicz, 1971).

Acrylic materials with more than one acrylate group began appearing in the 1960s. They act as cross-linking agents in the hardening of synthetic resins. Polyfunctional acrylic monomers, such as ethylene glycol dimethacrylate, have enjoyed wide use in dental reconstruction for several years (Winkler, Powell & Ortman, 1971; Bell, 1972). A bisphenol A based dimethacrylate, bis-GMA resin, has been used in dental sealants for over 10 years (Rock, 1977). The principal industrial use of these acrylic monomers has been in low energy curing ink and coating systems (Shahidi, Trebellas & Vona, 1974; Rybny, 1974; Shahidi & Powanda, 1975; Rybny, Trebellas & Vona, 1975).

PETA, TMPTA and HDODA have been assessed for their potential to irritate the skin and eye, using a standard test devised by Draize *et al.* (1944) (Anon.—Celanese Chemical Company). They were found to be moderately irritating for rabbit skin and severely irritating to the eye. These data suggest that irritation is possible in human use (Phillips *et al.*, 1972). PETA caused skin irritation in half of the humans exposed to it under a closed patch test for more than 0.95 days. This result infers that it will be a strong cutaneous irritant (Lanman, Elvirs & Howard, 1968). Under the conditions of use, both human and animal tests correctly predicted the irritant contact conjunctivitis and dermatitis that occurred.

Cases 10, 11, 12, 14, 15 and 16 were sensitized to PETA, based on the results of patch testing. All had handled PETA as a raw material. Men in various jobs with varying amounts of skin exposure were sensitized. The incidence was not greatly different in any job category.

Cases 14 and 15 experienced allergic contact dermatitis after MFA use in the plant was discontinued. The source of contact in these two men proved to be drums contaminated with PETA which had been stored for cleaning.

Case 10 experienced airborne allergic contact dermatitis. The contact may have been with vapourized monomer generated by the heat of ink grinding. Similar problems have occurred in the past with aliphatic amines used to harden epoxy resins (Dernehl, 1951; Bourne, Milner & Alberman, 1959).

In a plant similar to the one described in this study, seven persons developed allergic contact dermatitis due to MFAs (Emmett & Kominsky, 1975; Emmett & Kominsky, 1977). Allergy to PETA, HDODA and TMPTA was proven by patch tests. Four further cases of cutaneous MFA sensitization were subsequently documented (Emmett, in press).

Two commercial laboratories evaluated PETA for skin sensitization potential. One tested ten guinea-pigs using Buehler's technique (Buehler, 1965). The other utilized the guinea-pig maximization test (Magnusson & Kligman, 1970) with ten animals. Both laboratories reported that no animals were sensitized.

The results presented above for guinea-pig response to PETA confirm that it is a sensitizer. With an intradermal concentration of only 0.05%, the animals were sensitized. In a previous study of MMA (Nyquist, Koch & Magnusson, 1972) where a 0.15% concentration was used intradermally, no animals were sensitized, though in the latter instance only 0.15% concentration was used in the topical induction phase. It should also be noted that 5% MMA could be tolerated intradermally, and 100% topically, in the latter study, while only 0.05 and 10% respectively of PETA were tolerated. These findings suggest that PETA is more irritating and possibly a stronger sensitizer than the commonly used monomer MMA.

Seventy-eight percent of the nine human volunteers who were challenged with PETA reacted, and are presumed sensitized. This compares with Nyquist's experience with MMA, where 18 of 20 were sensitized to it by one 48 h patch test with a 5% solution of MMA (Nyquist *et al.*, 1972). In another investigation where MMA was applied as is to fifty human volunteers for 48 h, 20% were noted to be sensitized 10 days later (Speelman *et al.*, 1945). The difference in the sensitization rates between these two previously reported groups may be related to technique (Kligman, 1966), or to a difference in the sensitization potential of the two MMA products. Differences in sensitizing qualities of MMA from different MMA manufacturers have been documented (Magnusson, 1975). Though the size of the human study group is small, the fact that seven of nine subjects (78%) were sensitized is in keeping with these previous human experiments with acrylic monomer.

The guinea-pig maximization test has predicted human experimental and clinical sensitization by PETA. These results emphasize that animal predictive tests must be carried out with care if they are to be of value.

Methacrylate esters cross-react in sensitized guinea-pigs (Chung & Giles, 1977). MFAs also cross-react (Emmett, 1977). None of the seven patients sensitized to PETA, reported here, reacted to MMA. One other instance of lack of cross-reactivity with MMA has been reported (Emmett, 1978). Failure of cross-reaction between acrylic monomers has been reported previously (Jordon, 1975). MMA is probably not a useful chemical for the detection of MFA contact allergy.

Three cases of sensitization with epoxy acrylate resin have been reported previously (Emmett, 1978). None of the patients I patch tested with epoxy acrylate resins or linseed oil acrylate reacted. All of the affected patients from the ink plant have been able to return to work with no further skin problems in over one year of regular observation. The resins, being of higher molecular weight and lower relative chemical reactivity, appear to be less potent sensitizers.

The photoinitiators are essential components of the curing system. Esters of amyl-p-dimethylaminobenzoate used for this purpose have caused clinical photosensitivity in some ink manufacturing personnel (Emmett, Taphorn & Kominsky, 1977). No known instances of sun sensitivity occurred in this group of patients, although they had exposure to this material. This difference in incidence of clinical findings may reflect differences in hygiene or sun exposure, or both.

Beurey *et al.* (1976) have reported irritant and allergic contact dermatitis in printers exposed to

light-sensitive printing ink. The MFA tetraethylene glycol diacrylate was shown to be the cause of the skin reactions. I am reporting another instance of allergic contact dermatitis in a printer (Case 19) exposed to such inks, where the source of the patient's disease proved to be the multifunctional acrylate components PETA and TMPTA.

Fewer cases of skin reactions due to MFA have been reported in printers than in workers manufacturing light-sensitive inks. Contact with uncured ink containing free MFA is apparently less likely to induce allergy. I visited a printing plant using UV curing. Skin contact with the ink is infrequent, as these presses are automated and skin hygiene is better than in three ink manufacturing plants I examined. The lower frequency of skin contact and the low MFA concentration in the ink probably explain the difference in incidence of skin problems in these two working environments.

The cured ink is considered to contain little free MFA. The lack of skin reaction to the cured ink by Case 10, who was known to be sensitized, suggests this is true. The cured ink is thus unlikely to result in cutaneous problems in those handling it.

Contact with acrylic monomer in acrylic photo-engraving plates has resulted in allergic contact eczema (Rycroft, 1977). The nature of this process suggests that it may involve MFA technology. Patients suspected of having allergic contact dermatitis due to such materials should be assessed for cutaneous reactivity to MFAs in addition to MMA.

No cutaneous problems have been reported in dentists or patients exposed to ultraviolet-cured acrylic dental sealants which contain multifunctional acrylates. This may reflect the route of exposure and exposure time in the patients as well as the careful cutaneous hygiene of dentists.

MFAs are human irritants and sensitizers. The clinical and experimental data presented infer that PETA is as potent a sensitizer as MMA. MFAs probably can be handled without great cutaneous hazard as long as skin contact is minimized. Further instances of MFA allergy may occur in the future. Dermatologists should bear in mind that patch testing with MMA may fail to demonstrate sensitization in patients affected.

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REFERENCES

- ADAMS, R. (1969) *Occupational Contact Dermatitis*, p. 147. J.B. Lippincott, Philadelphia.
- ALLARDICE, J.T. (1967) Dermatitis due to an acrylic resin sealer. *Transactions of the St John's Hospital Dermatological Society*, **53**, 86.
- ANONYMOUS *Celanese Multifunctional Acrylate and Monomers in Conventional Coatings and Adhesives*. Celanese Chemical Company, New York.
- BELL, A. (1972) The acrylics. *New York Journal of Dentistry*, **42**, 324.
- BEUREY, J., MONGEOLLE, J.-M. & WEBER, M. (1976) Accidents cutanés des résines acryliques dans l'imprimerie. *Annales de Dermatologie et de Syphiligraphie*, **103**, 423.
- BOURNE, L., MILNER, F. & ALBERMAN, K. (1959) Health problems of epoxy resins and amine curing agents. *British Journal of Industrial Medicine*, **16**, 81.
- BUEHLER, E. (1965) Delayed contact hypersensitivity in the guinea-pig. *Archives of Dermatology*, **91**, 171.
- CARESS, A. (1944) 'Perspex': Methyl methacrylate polymer. *Endeavour*, **3**, 156.
- CHUNG, C. & GILES, A. (1977) Sensitization potential of methyl, ethyl and n-butyl methacrylate and mutual cross-sensitization in guinea-pigs. *Journal of Investigative Dermatology*, **68**, 187.
- CRISSEY, J. (1965) Stomatitis, dermatitis and denture materials. *Archives of Dermatology*, **92**, 45.
- DERNEHL, C. (1951) Clinical experiences with exposure to ethylene amines. *Industrial Medicine and Surgery*, **20**, 541.
- DRAIZE, J., WOODARD, G. & CALVERY, H. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *Journal of Pharmacology and Therapeutics*, **82**, 377.
- DUNN, H., BURACHINSKY, B., ELY, J. & GRUBEL, P. (1966) 'Inks'. In: *Encyclopedia of Chemical Technology* (Ed. by R.E. Kirk and D.F. Othmer), Vol. 611. John Wiley and Sons, New York.

- EMMETT, E. & KOMINSKY, J. (1975) *Health Hazard Evaluation Determination Report No. 75-106-247*. U.S. Department of Health Education and Welfare, National Institute of Occupational Safety and Health, Cincinnati.
- EMMETT, E. & KOMINSKY, J. (1977) Allergic contact dermatitis from ultraviolet cured inks. *Journal of Occupational Medicine*, **19**, 113.
- EMMETT, E. (1977) Contact dermatitis from polyfunctional acrylic monomers. *Contact Dermatitis*, **3**, 245.
- EMMETT, E., TAPHORN, B. & KOMINSKY, J. (1977) Phototoxicity occurring during the manufacture of ultraviolet cured ink. *Archives of Dermatology*, **113**, 770.
- FISHER, A., FRANKS, A. & GLICK, H. (1957) Allergic sensitization of the skin and nails to acrylic plastic nails. *Journal of Allergy*, **28**, 84.
- FISHER, A. (1973) *Contact Dermatitis*, 2nd edn, p. 185. Lea and Febiger, Philadelphia.
- FREGERT, S. (1974) *Manual of Contact Dermatitis*, p. 31. Munksgaard, Copenhagen.
- FREGERT, S. & HJORTH, N. (1972) The principal irritants and sensitizers. In: *Textbook of Dermatology* (Ed. by A. Rook, D. Wilkinson and F. Ebling), 2nd edn, Vol. 2, p. 1893. Blackwell Scientific Publications, Oxford.
- GOODWIN, P. (1976) Onycholysis due to acrylic nail applications. *Clinical and Experimental Dermatology*, **1**, 191.
- HARRIS, D.K., (1953) Health problems in the manufacture and use of plastics. *British Journal of Industrial Medicine*, **10**, 255.
- JENNY, J., EVANS, L. & RAKER, C. (1965) Hoof repair with plastics. *Journal of the American Veterinary Medical Association*, **147**, 1340.
- JORDON, W. (1975) Cross-sensitization patterns in acrylate allergens. *Contact Dermatitis*, **1**, 13.
- KLIGMAN, A. & WOODING, W. (1967) A method for the measurement and evaluation of irritants on human skin. *Journal of Investigative Dermatology*, **49**, 78.
- KLIGMAN, A. (1966) The identification of contact allergens by human assay III. The maximization test: A procedure for screening and rating contact sensitizers. *Journal of Investigative Dermatology*, **47**, 393.
- LANMAN, B., ELVERS, W. & HOWARD, C. (1968) The role of human patch testing in a product development program. *Proceedings, Joint Conference on cosmetic sources, Toilet Goods Association*, p. 135. The Toilet Goods Association, Inc. Washington, D.C.
- LAVORGNA, J., BURNSTEIN, N., SCHILLER, A. & HARRIS, W. (1972) The carcinogenesis of plastics used in orthopaedic surgery. *Clinical Orthopaedics and Related Research*, **88**, 223.
- MAGNUSSON, B. & KLIGMAN, A. (1970) *Allergic Contact Dermatitis in the Guinea-pig*. Charles C. Thomas, Springfield, Illinois.
- MAGNUSSON, B. & MOBACKEN, H. (1972) Contact allergy to a self-hardening acrylic sealer for assembling metal parts. *Berufsdermatosen*, **20**, 198.
- MAGNUSSON, B. & MOBACKEN, H. (1972) Allergic contact dermatitis from acrylate printing plates in a printing plant. *Berufsdermatosen*, **20**, 138.
- MAGNUSSON, B. (1975) The relevance of results obtained with the guinea-pig maximization test. In: *Animal Models in Dermatology* (Ed. by H. Maibach), p. 76. Churchill Livingstone, New York.
- NYQUIST, G., KOCH, G. & MAGNUSSON, B. (1972) Contact allergy to medicaments and materials used in dentistry (III). *Odontologisk Revy*, **23**, 197.
- PEGUM, J. & MEDHURST, F. (1971) Contact dermatitis from penetration of rubber gloves by acrylic monomer. *British Medical Journal*, **ii**, 141.
- PHILLIPS II, L., STEINBERG, M., MAIBACH, H. & AKERS, W. (1972) A comparison of rabbit and human skin response to certain irritants. *Toxicology and Applied Pharmacology*, **21**, 369.
- ROCK, W. (1977) Fissure sealants—results of a three year clinical trial using an ultraviolet sensitive resin. *British Dental Journal*, **142**, 16.
- ROUTLEDGE, R. (1971) Contact dermatitis and bronchial irritation due to ammonium acrylate in the motor industry. *Transactions of the Society of Occupational Medicine*, **21**, 59.
- RYBNEY, C. (1974) Ultraviolet radiation cured coatings. *Journal of Paint Technology*, **45**, 61.
- RYBNEY, C., TREBELLAS, J. & VONA, J. (1975) New raw materials for radiation curable coatings. *Technical paper FC 75-305*, Society of Manufacturing Engineers, Dearborn, Michigan.
- RYCROFT, R. (1977) Contact dermatitis from acrylic compounds. *British Journal of Dermatology*, **96**, 685.
- SHAHIDI, I. & POWANDA, T. (1975) Ultraviolet Curing: A review of the technology. *American Ink Maker*, **53**, 20.
- SHAHIDI, I., TREBELLAS, J. & VONA, J. (1974) Multifunctional monomers for UV cure. *Paint and Varnish Production*, **64**, 32.
- SPEALMAN, C., MAIN, R., HAAG, H. & LARSON, P. (1945) Monomeric methyl methacrylate. *Industrial Medicine*, **14**, 292.
- SPIECHOWICZ, E. (1971) Experimental studies on the effect of acrylic resin on rabbit skin. *Berufsdermatosen*, **19**, 132.
- WINKLER, S., POWELL, J. & ORTMAN, H. (1971) Ultraviolet absorbers in dental monomers. *Dental Digest*, **77**, 460.

The effects of mental and physical stress on the incidence of skin disorders

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SUMMARY

The effect of civil unrest and terrorism in Northern Ireland from 1969 on skin diseases which are generally accepted to be aggravated by stress and strain has been studied (1) by reporting cases making claims against the government for skin diseases developed as the result of violence and (2) studying changes in the pattern of skin diseases at skin clinics in Northern Ireland over the last 10 years. There has been little change in the pattern of skin diseases which could be attributed to mental or physical stress.

The effects of stress, strain, anxiety and tension on the skin in general and various skin diseases in particular have always been controversial. There are some skin diseases which are generally accepted as being aggravated by stress and strain, such as lichen simplex (lichen simplex chronicus of Vidal) and psoriasis. There are others, however, where the association is disputed, such as alopecia areata, seborrhoeic eczema, urticaria, rosacea and vitiligo. These factors are difficult to assess in an objective way but Kidd & Meenan (1961) did not find that there was any increase in so called nervous skin disease in the population of a mental hospital.

As is well known, Northern Ireland has been subjected to civilian disturbances since 1969, having previously been a more than usually peaceful country. Due to the civil disturbances many people have suffered acute stress and anxiety (Lyons, 1974). This emotional strain is due to a combination of factors including sectarianism, daily bombings of property and consequent injury and loss of life, intimidation, the loss of relatives and friends and, in general, the continuous stress and anxiety of terrorism. To quote Lenin, 'The object of terrorism is to terrorise', and more recently Clutterbuck has said, 'Kill one and terrorise ten thousand'. No real parallel can be drawn between the reactions of soldiers and civilians. The soldier is away from his home and family, he may be in the army involuntarily and often has to undergo severe physical discomfort and fatigue. Other differences include the effect of training, habitual exposure to danger and the virtual lack of freedom of independent action. These factors, however, have not usually acted on the civilian to the same extent, the danger of life and property being the main stress for him (Lyons, 1971). There has been a significant decrease in depressive illness in Belfast in both sexes and all age groups since the onset of the present terrorist campaign. This has been more pronounced in males but the decrease is confined to those in social

groups IV and V. The decrease is more significant in riot areas. The suicide rate has fallen by almost 50% (Table 1) and there is a noticeable increase in the rates of homicide and crimes of violence, due almost exclusively to terrorism. In contrast the rural country shows a sharp increase in male depressives (Lyons, 1972).

TABLE 1. Suicide rates per 100,000 population in Northern Ireland

	Male	Female
1965	5.9	3.7
1966	6.5	4.6
1967	8.8	4.4
1968	7.0	6.2
1969	7.3	4.9
1970	4.7	3.2
1971	4.5	2.4
1972	4.0	2.0
1973	4.2	4.9
1974	5.1	2.9
1975	4.7	2.6

The situation in Northern Ireland is unique in one important aspect, namely, the social and hospital services have continued to work normally in spite of the civil disturbances—unlike a state of war. Northern Ireland is part of the United Kingdom, has a National Health Service and medical consultations in hospitals are free to the entire population, so that our figures of hospital records for each year are dependable. In spite of the recent troubles Northern Ireland still has one of the lowest fatal accident rates of any country in the world and very much lower death rates from violence than most of the American cities (Bates, 1974).

It was therefore felt that it might be useful to see if there was any increase in skin disease which may be thought to have an emotional background, or if there was any change in the pattern of skin disease in our population. This can be assessed in two ways:

(1) Any person who suffers physical injury as a result of a civil disturbance in Northern Ireland, for example in a shooting or a bomb explosion, can make a claim against the Government for damages. Skin diseases resulting from distressing experiences come under this heading. The following patients were seen by us for medico-legal examination in the one year up to June 1976:

Psoriasis	5
Prurigo nodularis	1
Seborrhoeic eczema	1
Lichen simplex	2
Lichen simplex and seborrhoeic eczema	1
Urticaria and dermographism	3
Alopecia areata	1
Alopecia areata and seborrhoeic eczema	1
Rosacea	1
Total	16

In each of these patients we accepted that their skin condition had been brought about or influenced by stress. The surprising thing for us is that there were so few. The details of these patients are as follows:

G.O'N. (aged 26)

He noticed men plant a car bomb outside a public house where his father was drinking. He ran over to warn his father but when he was 50 yards away from the pub the car exploded. He was knocked unconscious, his father and his mother were both injured and 5 days later he developed psoriasis for the first time.

There was one other patient who developed psoriasis for the first time following severe mental stress and 3 patients who suffered a severe exacerbation of psoriasis following emotional and/or physical stress.

G.W. (aged 24)

He was coming out of a club, was attacked, received multiple gunshot wounds to his hands, had his nails removed and was further physically assaulted. After this he developed prurigo nodularis on the fingers of the right hand.

Mrs M.L. (aged 50)

This lady who lives alone, being separated from her husband, was caught in two explosions near her home and after this became very nervous and developed lichen simplex on both forearms.

There was one other similarly affected patient.

J.McC. (aged 26)

He was taken at gunpoint to the firm at which he worked where the gunmen removed some chemicals from his firm. He himself was tied up and left; a month later he developed generalized urticaria.

There was one other similarly affected patient.

J.S.McC. (aged 50)

He was a part-time fireman dealing with a fire in a building when a second bomb went off within the building. He received severe hand and abdominal injuries and one month later developed alopecia areata and seborrhoeic eczema.

Mrs H.S. (aged 31)

An explosion occurred in her garage. She was very frightened and developed a habit of rubbing her hands together. She has always had dermographism and now she has almost constant dermographism on her hands.

A.M. (aged 21)

He was sitting 4 or 5 yards away when a 30 lb bomb exploded. He received multiple lacerations and 2 days later developed seborrhoeic eczema.

T.M. (aged 49)

He was having a drink in a public house which was bombed and he received multiple severe injuries. Within 2 weeks he developed six quite large areas of alopecia areata.

Mrs B.O'D (aged 62)

A bomb was thrown into her house, her husband was killed, and she was struck by flying glass but was not badly injured. Three months later and for the first time she developed severe rosacea.

C.C. (aged 25)

This was a policeman who was in a house when a bomb went off in a public house next door. He was severely shocked but not physically injured. Just after this he developed both lichen simplex and seborrhoeic eczema.

(2) In Northern Ireland for many years a diagnostic index has been kept of skin diseases of all patients reporting to all skin clinics in the province. For the most part there is a very short waiting list or perhaps even none at all for these clinics and the clinics are so situated throughout the country that in no case does a patient have to travel more than 20 miles to be seen by a consultant dermatologist.

TABLE 2. Violence, explosions and referral of new patient per 100,000 population to all skin clinics in Northern Ireland

	Total number of deaths due to violence	Total number of explosions	Hyperhidrosis		Lichen simplex		Generalized pruritus		Psoriasis	
			M	F	M	F	M	F	M	F
1965	0	0	2.1	2.1	14.1	17.0	3.1	4.1	43.3	62.2
1966	0	0	1.9	1.7	10.5	12.4	2.1	3.4	46.9	55.9
1967	0	0	2.2	1.3	10.2	15.0	2.1	3.3	46.1	59.9
1968	0	0	3.0	2.7	11.9	12.9	2.3	4.3	47.2	59.8
1969	13	8	3.1	1.8	9.8	12.4	4.6	5.8	51.5	65.7
1970	25	155	3.1	1.4	7.6	10.6	2.3	7.7	52.8	67.9
1971	173	1033	3.4	2.7	8.9	11.0	4.9	8.3	59.2	64.4
1972	467	1495	4.8	2.9	8.5	9.8	6.7	7.0	59.9	73.2
1973	250	1007	5.0	2.3	6.5	7.5	6.5	10.4	52.1	64.6
1974	216	669	3.5	3.1	5.7	8.3	6.3	9.5	65.0	79.1
1975	247	366	5.1	4.6	5.9	4.2	6.7	9.4	74.4	83.8

TABLE 3. Kendall rank correlation coefficients between level of disease and levels of violence (see text for definitions of these levels)

Disease	Males		Females	
	Deaths	Explosions	Deaths	Explosions
Common warts	0.000	0.079	0.156	0.311
Basal cell epitheliomata	-0.392	-0.436	-0.351	-0.268
Atopic dermatitis	0.344	0.177	0.424	0.263
Urticaria	0.327	0.449	0.566	0.404
Hyperhidrosis	0.871†	0.697†	0.308	0.205
Lichen simplex	-0.693†	-0.575*	-0.748†	-0.626†
Generalized pruritus	0.822†	0.695†	0.796†	0.674†
Pruritus ani	-0.491*	-0.491*	0.616†	0.513*
Rosacea	-0.043	-0.128	-0.020	0.182
Seborrhoeic eczema	-0.506*	0.428	0.059	0.098
Lichen planus	0.283	0.370	0.237	0.366
Psoriasis	0.706†	0.628†	0.320	0.240
Alopecia areata	-0.580*	-0.540*	-0.505*	-0.586*

*Significant at $P < 0.05$; significant at $P < 0.01$.

We compared the level of various skin diseases with the level of violence in Northern Ireland over the past 11 years. We included two diseases which are unlikely to be influenced by violence, namely, common warts and basal cell epitheliomata. We also included a number of conditions which are thought by some people to be influenced by emotional factors.

The level of each disease was assessed by expressing the number of new attendances for that disease in a given year as a percentage of the total number of new attendances in that year. The level of violence in each year has been assessed in two ways, firstly by considering the number of deaths and secondly by considering the number of explosions (figures kindly provided by the Royal Ulster Constabulary) (see Table 2). For each disease separately, Kendall rank correlation coefficients (Kendall, 1948) were calculated to determine if the level of disease in a given year was correlated with either of the two available levels of violence for that year. These coefficients are given in Table 3. A positive coefficient indicates that level of disease increases with increasing level of violence and a negative coefficient that disease level increases with decreasing level of violence. Each coefficient was tested against a hypothetical value of zero (i.e. no association between level of disease and level of violence). Those coefficients which are large enough to suggest a real association, i.e. significantly different from zero at $P < 0.05$, are indicated in Table 2.

The coefficients in Table 3 are not always consistent between the sexes. For example, the disease level of pruritus ani is negatively correlated with each level of violence for males but positively correlated for females. On the other hand consistent results were obtained for lichen simplex, generalized pruritus and psoriasis.

One must remember certain points about this study.

- (1) The patients included in this study form only the tip of the iceberg—the majority of patients being seen by their family doctors.
- (2) An increasing level of violence may well cause a decrease in hospital attendances because patients are reluctant to travel. This could be specially true for conditions of a more trivial nature.
- (3) Annual disease level has been correlated with annual level of violence. Because of this, the coefficients of Table 3 are based on only eight pairs of values so that only numerically large coefficients will be detected as significantly different from zero.

It is noteworthy that the incidence in numbers of patients with lichen simplex fell to less than half between the years 1965 and 1975.

We did not examine the ratio of new cases to population at risk, mainly because during the period under study new clinics were being opened which led to an overall increase in the number of attendances. It might be argued that in considering years rather than months, or weeks, the time lag between violence and its effect was too long. However, for many of the diseases subdivision by month of attendance would have resulted in small numbers.

One might explain the increase in hyperhidrosis by the awareness of the family doctors of surgical treatment for axillary hyperhidrosis. The increase in generalized pruritus could be explained by the special interest of one of us in this condition at the time (Beare, 1976). The increase in cases of psoriasis is quite possibly due to the result of the overuse of potent steroid preparations and the resultant rebound phenomenon.

CONCLUSION

The small number of patients who developed skin disease after suffering from violence is striking. We

believe that our second study shows that there has been very little change in the incidence of skin diseases which may have an emotional element in aetiology during the period 1965-1975 when our country suffered greatly from widespread terrorism.

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REFERENCES

- BATES, D.R. (1974) Practising science in Northern Ireland. *Nature*, **250**, 754.
BEARE, J.M. (1976) Generalized pruritus. A study of 43 cases. *Clinical and Experimental Dermatology*, **1**, 343.
KENDALL, M.G. (1948) *Rank Correlation Methods*. Griffin and Co. Ltd., London.
KIDD, C.B. & MEENAN, F.C. (1961) A dermatological survey of longstay mental patients. *British Journal of Dermatology*, **73**, 129.
LYONS, H.A. (1971) Psychiatric sequelae of the Belfast riots. *British Journal of Psychiatry*, **118**, 265.
LYONS, H.A. (1972) Depressive illness and aggression in Belfast. *British Medical Journal*, **1**, 342.
LYONS, H.A. (1974) Terrorists bombing and the psychological sequelae. *Journal of the Irish Medical Association*, **67**, 15.

Percutaneous absorption of phenol and methyl alcohol in Magenta Paint B.P.C.

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SUMMARY

Estimations of phenol and methyl alcohol in the urine of 16 infants with seborrhoeic eczema treated with Magenta (Castellani's) Paint B.P.C. were made to determine the degree of absorption. Phenol was detected in four cases and methyl alcohol in none.

For many years Magenta Paint B.P.C. has been used in the Royal Belfast Hospital for Sick Children (RBHSC) as the treatment of choice in infants with seborrhoeic eczema. The napkin area and skin folds are painted with this preparation twice daily for 48 h. The treatment is then changed to cream or ointment containing nystatin.

It had been observed that children with a fair complexion or to whom the paint had been applied shortly after bathing became mildly shocked and drowsy.

CASE HISTORY

In March, 1976, a 6-month-old infant with excessive seborrhoeic eczema affecting the scalp, face, skin folds and napkin area was admitted to the Skin Ward (RBHSC) for treatment. The child's entire body, apart from the face, was covered in Magenta Paint. Twelve hours later the child was drowsy, had shallow respirations and was passing blue urine. No further painting was carried out and she recovered spontaneously.

It was decided to investigate seborrhoeic infants treated with Magenta Paint for absorption of phenol and methyl alcohol, as these substances could account for the central nervous depression observed.

PATIENTS AND METHODS

Sixteen infants with seborrhoeic eczema, aged between 2 and 5 months, were painted twice daily for 48 h in the napkin area and skin folds. Approximately 11–15% of the body surface was painted and an average of 32 ml of paint was applied to each child. The children were nursed without any dressings. A 24-h urine collection was then made by means of a plastic bag taped to the child's perineum. Every effort was made to avoid contamination of the urine with the Magenta Paint.

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Laboratory investigations

The 24 h specimen of urine was examined for phenol and methyl alcohol. The former was estimated by the semi-quantitative method of Rainsford & Lloyd Davies (1965) and the latter by means of gas liquid chromatography.

As phenol and methanol are known hepatotoxins, blood from eight of the children was taken for liver function studies. Blood urea and electrolyte values were also estimated as methanol and phenol are excreted by the kidneys, the latter in the form of benzenesulphonates and glucuronates (upon oxidation these substances tint the urine green, which would account for the urinary discolouration in our first case). In addition, blood from 6 children with seborrhoeic eczema not painted with Magenta Paint was examined as was blood from 4 controls.

RESULTS

Phenol was detected in the urine of 4 children. Methyl alcohol was present in none.

Serum bilirubin was normal in the eight cases examined. Serum alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase and creatine kinase showed a wide range of values but these varied no more in the painted, unpainted or control groups.

Serum electrolyte values were normal in the eight painted infants. Blood urea was elevated at 8.5 mmol/l and 9.7 mmol/l (normal 2.2–5.8) in two. Neither of these children had phenol in their urine. However, the blood urea was also elevated at 8.4 mmol/l in one of the unpainted seborrhoeics, so the significance of the two raised values is doubtful.

DISCUSSION

The fact that phenol was detectable in the urine of some of the infants treated with Castellani's paint taken in conjunction with the symptoms our first case had developed with extensive painting made us hesitant to collect a large series of cases. We felt, however, that from our observations and findings there was a lesson to be learned. All too often substances are applied to the skin without sufficient consideration of the constituents and possible side effects through absorption. Magenta paint is usually applied to intertriginous areas of the skin or to leg ulcers for its drying ability and its anti-yeast and anti-bacterial actions. Such areas are ideal for absorbing chemicals present in the paint. When one studies its composition (see below) it is found to contain a formidable list of substances.

Magenta paint has been withdrawn from production as carcinogenic compounds are involved in its synthesis.

Magenta Paint, B.P.C. *m* Formula

Magenta	0.4 g
Phenol	4.0 g
Boric acid	0.8 g
Resorcinol	8.0 g
Acetone	4.0 ml
Industrial methylated spirit (90%)	8.5 ml
Water	to 100 ml

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REFERENCE

- RAINSFORD, S.G. & LLOYD DAVIES, T.A. (1965) Urinary excretion of phenol by men exposed to vapour of Benzene; a screening test. *British Journal of Industrial Medicine*, **22**, 21.

Skin reactions caused by vitamin K in patients with liver disease

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SUMMARY

Vitamin K and its analogues are frequently used in treatment of the hypoprothrombinaemia found in disease of the liver, biliary tract and small intestine. Most cases of cutaneous toxicity to vitamin K have been described in the French literature, but only two cases from Britain. This paper reports six patients with chronic liver disease who developed cutaneous reactions around the site of injection of vitamin K, and the results of investigations to further understanding of the pathogenesis of the rash.

PATIENTS

Table 1 summarizes the clinical features and the relevant investigations on the six patients. The rashes were similar in all patients (Table 2), occurring at the site of vitamin K injections (Konakion, Roche) 7–16 days after the initiation of therapy which had been given for a raised prothrombin time. The striking feature seen in four patients was the fact that the rash spread, either towards the sacrum or more usually down to the thigh, over a period of 2–5 days (Fig. 1). Resolution was with scaling within 9–22 days. The amount of oil soluble vitamin K (Konakion) given before the onset of the rash varied between 270 and 440 mg. No patient had previously received Konakion and in all patients at the time of onset of the rash the prothrombin time was not responding satisfactorily to treatment with Konakion. After stopping oil soluble vitamin K, 5 patients were given a water soluble vitamin K analogue (Synkavit, Roche) with no adverse reaction.

INVESTIGATIONS

The relevant diagnostic investigations are shown in Table 1. In addition, skin tests were performed in 5 patients. Within one week of development of the rash, two patients (Cases 2 and 3) were skin tested by intradermal injection of 0.1 ml of the vitamin K preparations vitamin K₁, phytomenadione (Konakion, 10 mg/ml Roche) and menadiol sodium diphosphate (Synkavit, 10 mg/ml, Roche) and the components of Konakion, Cremophor EL, propylene glycol and phenol.

In both cases induration and erythema occurred, maximal at 24–72 h, at the site of injection of

This work was carried out in the Department of Medicine, St James's Hospital.

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TABLE 1

Diagnosis:	Case no.					
	I	2	3	4	5	6
	Chronic active hepatitis	Chronic active hepatitis	Chronic active hepatitis, rheumatoid arthritis	Chronic venous congestion of liver, congestive heart failure, rheumatoid arthritis	Alcoholic cirrhosis	Congenital hepatic fibrosis
Sex	F	F	F	F	F	F
Age	17	24	39	50	48	12
Prothrombin time: (secs) Control 12s.	24	17	19	21	24	19
Liver biopsy	Yes	Yes	Yes	Yes	No	Yes
A.N.F.:	Positive	Positive	Negative	Negative	Negative	Negative
L.E. Cells:	Negative	Positive	Negative	Negative	Negative	Negative
Smooth muscle antibody	Positive	Positive	Negative	Negative	Negative	Negative
Mitochondrial antibodies	Negative	Positive	Positive in low titre	Negative	Negative	Negative
Hepatitis B. antigen	Negative	Negative	Negative	Negative	Negative	Negative
Other therapy	Ampicillin Cloxacillin Tetracycline	Nil	Potassium Chloride Paynocil	Prednisolone	Spiro- lactone Potassium chloride Folic acid Ascorbic acid α -Tocopherol Cotrimoxazole	Nil

TABLE 2

	Case no.					
	1	2	3	4	5	6
Erythematous	Yes	Yes	Yes	Yes	Yes	Yes
Indurated	Yes	Yes	No	Yes	No	No
Purpuric	No	No	No	Yes	No	No
Mild irritation	No	No	Yes	Yes	Yes	Yes
Spread	Yes	No	Yes	No	Yes	Yes
Day of onset of rash	13	9	9	16	10	7
Total dosage of Konakion (mg)	340	360	270	440	300	280
Duration of rash (days)	11	9	19	18	22	9

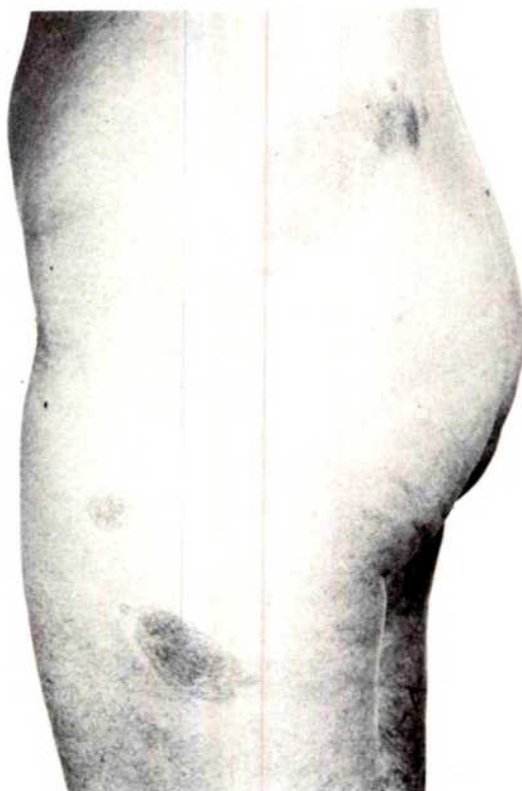


FIGURE 1. Spreading of the rash to the thigh. Injection sites in the upper and outer quadrant of the buttock only.

Konakion. From 24 h to 15 days after skin testing there was pruritus at the site of Konakion injections. The reactions resolved within 20 days. There was no significant skin reaction at the site of injection of Synkavit or the components of Konakion.

In one patient (Case 2) lymphocyte transformation was not stimulated by up to 200 µg/ml of Konakion, nor was leukocyte migration inhibited by 500 µg/ml Konakion. Skin biopsy of the lesion showed a non-specific perivascular infiltration with chronic inflammatory cells in the dermis; staining for immunoglobulins G, A, M and E, and the C₃ component of complement gave negative results.

Three patients (Cases 1, 5 and 6) were skin tested several months after the development of the rash. Erythema and induration with pruritus occurred at the site of Konakion injection in all three. The reactions resolved within two weeks. There was no reaction to Synkavit or the components of Konakion.

Skin testing with intradermal injection of 0.1 ml of Konakion was also performed on ten other patients with chronic liver disease, all of whom had previously received intramuscular Konakion without developing skin reactions, and ten healthy volunteers, not previously exposed to Konakion.

In 4 out of 10 patients with liver disease, intradermal Konakion caused erythema, induration and pruritus, which was present at 48 hours after injection and resolved over the following 10 days. Erythema and induration also occurred at 48 hours in 2 out of 10 healthy volunteers. Two healthy volunteers had no early reaction to Konakion, but after 6 days developed pruritus, erythema and

urticaria at the injection site; the presence of these features fluctuated, but they resolved over the following week. In one volunteer, following a positive skin reaction to Konakion, a further intradermal injection caused immediate erythema, progressing over 5 days to a 52.5 cm² area of induration with local pruritus and urticaria recurring over short periods; the reaction resolved completely over the next four weeks. However, the positive skin tests in the control subjects and other patients with liver disease were less frequent and usually less florid than in patients with the skin rash.

DISCUSSION

Fewer than 50 cases of localized skin reactions to vitamin K have been reported, mainly from France. The six cases we have described are similar to those described by Piguet & Bertheuil (1964) and subsequently by Carton (1965), Texier *et al.* (1972) and Barnes & Sarkany (1976).

The rash occurs at the site of the injection within a few days to several weeks after initiation of treatment. It is always erythematous, sometimes indurated and sometimes mildly itchy. A diagnostic feature is its tendency to spread down the thigh. The rash was colourfully described by Texier *et al.* (1972) as being distributed like the 'ceinturon de cow-boy avec ses revolvers'. The spreading phenomenon may be due to the injection fluid tracking along fascial planes. In most cases the rash resolved over a period of 2–12 weeks but Texier *et al.* (1974) reported that 11 cases developed a sclerodermatous appearance at the site of the injection and Larregue *et al.* (1975) described a further case of lumbar scleroderma. Our patients, who received far less vitamin K than the patients of Texier *et al.* (1972), have shown no evidence of scleroderma after follow-up for 1–4 years.

The pathogenesis of this condition is not clear. Several possibilities exist. Texier *et al.* (1972) suggested that the presence of concomitant liver disease is important, but if this is so, the reaction is not related to either the nature of the liver disease or its severity. Vitamin K is also used in the hypoprothrombinaemia of obstructive jaundice and malabsorption, but the response of the prothrombin time in these cases is usually rapid and complete and thus only patients with liver disease are likely to be given large intramuscular doses of vitamin K. The dose of vitamin K is probably important; most of the reported cases had greater than 100 mg before developing the skin rash; the cases developing sclerodermatous changes had 500 mg or more, and sometimes massive doses of more than 2500 mg.

There can be no doubt that the skin reaction was due to the vitamin K preparation; there was no correlation between the skin reaction and other drugs administered to either our or other reported cases. The reactions were not related to the use of a single batch of vitamin K, as they appeared at intervals over the last four years, when different batches were in use. Vitamin K is available as a water soluble or oil soluble preparation. In our cases the reaction occurred only to the oil soluble preparation. This was confirmed by negative skin tests to water soluble vitamin K and the absence of adverse reactions on continuing treatment with a water soluble preparation, Synkavit (Roche).

Reactions to systemically administered vitamins are unusual (Høvdning, 1968) and are sometimes due to additives. Texier *et al.* (1974) concluded that the reaction in his patients was due to the phenol added as a preservative. However, skin testing in our patients revealed no evidence that the reactions were directly caused by the phenol, propylene glycol or Cremophor EL, which is a polyethoxylated castor oil. Some non aqueous solvents, including vegetable oils, are known to cause allergic reactions, and fixed oils have caused local tissue reactions such as cysts, foreign granulomas and occasionally nerve injury (Speigel & Noseworthy, 1963).

Piguet & Bertheuil (1964) found positive intradermal reactions with a whole vitamin K₁ preparation, but not with pure vitamin K₁, or the solvent alone. However, Heydenreich (1977) described a transient positive skin test with pure vitamin K₁, but not with the solvent. It would have been of interest to have repeated the intradermal tests in our patients with different combinations of the individual

components as this may modify the physical or chemical structure to produce an adjuvant; for ethical reasons this was not possible.

A precise description of intradermal tests is not available from all reports, but in most the reaction consisted of itching, erythema and induration with a peak response at between 2 and 5 days, and lasting for up to 8 days, although in those with scleroderma induration persisted for much longer. This description, however, does not tell us whether the inflammatory response was immunological or non-immunologically mediated. There is no histological confirmation in any of the patients' intradermal tests. One of our patients had a biopsy of a clinical lesion and the histology was suggestive of a non-immunological mechanism. Texier *et al.* (1974) performed intradermal tests in controls and in patients with liver disease; their data suggested that liver disease was an essential factor for the reaction to occur. However, our results suggest that skin reactions to intradermal injection of vitamin K are not confined to those with liver disease, and neither is the presence of liver disease invariably associated with a positive skin test. The results of intradermal tests may not, of course, be applicable to the reactions occurring after therapeutic doses given intramuscularly.

Lymphoblastic transformation tests by Texier *et al.* (1972) and ourselves gave no evidence of an immune mechanism, and we found no evidence of leukocyte migration inhibition with vitamin K.

In summary, we have described six patients with a local reaction to oil soluble vitamin K; the pathogenesis of this reaction remains uncertain, but we suggest that large doses of vitamin K are implicated, and that the reaction may not be immunologically determined.

REFERENCES

- BARNES, H.M. & SARKANY, I. (1976) Adverse skin reaction from Vitamin K₁. *British Journal of Dermatology*, **95**, 653.
- CARTON, M.F.-X. (1965) Reaction allergique au cours d'un traitement: vitamine K₁ + extrait de foie. *Bulletin de la Société française de dermatologie et de syphiligraphie*, **72**, 228.
- HØVDING, G. (1968) Anaphylactic reaction after injection of vitamin B₁₂. *British Medical Journal*, **iii**, 102.
- HEYDENREICH, G. (1977) A further case of adverse skin reaction from vitamin K₁. *British Journal of Dermatology*, **97**, 697.
- LARREGUE, M., GALLET, Ph., DE GIACOMONI, P. & RAT, J.P. (1975) Sclerodermie lombo-fessiere consecutive a des injections de vitamine K₁. *Bulletin de la Société française de dermatologie et de syphiligraphie*, **82**, 447.
- PIGUET, M.M.B. & BERTHEUIL, F. (1964) Accidents cutanes allergiques provoques par une preparation injectable de vitamine K₁ synthetique. *Bulletin de la Société française de dermatologie et de syphiligraphie*, **71**, 486.
- SPIEGEL, A.J. & NOSEWORTHY, M.M. (1963) Use of non-aqueous solvents in parenteral products. *Journal of Pharmaceutical Sciences*, **52**, 917.
- TEXIER, L., GAUTHIER, Y., GAUTHIER, O., SURLEVE BAZEILLE, J.-E. & BOINEAU, D. (1974) Sclerodermies lombo-fessiers consecutives a des injections intramusculaires de vitamine K₁. *Bordeaux Medical*, **10**, 1571.
- TEXIER, L., GENDRE, Ph., GAUTHIER, O., GAUTHIER, Y., SURLEVE-BAZEILLE, J.-E. & BOINEAU, D. (1972) Hypodermes sclerodermiformes lombo-fessieres induites par des injections medicamenteuses intramusculaires associees a la vitamine K₁. *Annales de Dermatologie et de Syphiligraphie*, **99**, 363.

Case Reports

Wiskott–Aldrich syndrome with partial response to transfer factor

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SUMMARY

A male infant presented with dermatitis, purpura and susceptibility to bacterial infections. The clinical diagnosis of Wiskott–Aldrich syndrome was confirmed and after full immunological assessment, treatment with transfer factor was commenced. This has resulted in a rise in the platelet count and improvement in the bleeding tendency. This improvement in the haematological aspect of the disease has, however, been accompanied by exacerbations of the cutaneous lesions.

In 1937 Wiskott reported a family of three brothers with the triad of dermatitis, purpura and eczema, all of whom died within the first 5 years of life, and in 1954 Aldrich reported a family with three generations affected by a similar disorder (Aldrich, Steinberg & Campbell, 1954). A sex linked recessive inheritance for the disorder was postulated and it has become eponymously known as the Wiskott–Aldrich syndrome. The three constant features of this condition are thrombocytopenia, eczema, and undue susceptibility to bacterial infections. Other features reported with varying frequency are diarrhoea, low levels of serum IgM, variable levels of serum IgA, and depressed cell mediated immune function *in vitro*. The case here reported is of interest in that while response to administration of transfer factor (TF) in the form of improved resistance to infection has previously been reported, there are no reports in the literature of such a clear relationship between TF administration and a rise in platelet count as is demonstrated in this case.

CASE REPORT

A male infant first presented at the age of 8 weeks with erythematous scaling cutaneous lesions involving napkin area, scalp, arms and legs. At the time of presentation he was receiving only breast milk and artificial feeding was not introduced until he was 4 months old. He was the second child of healthy parents, the older child being female, and there was no parental consanguinity. The skin lesions responded rapidly to topical therapy and he was discharged from the dermatology clinic.

Eleven months later he presented again with intensely pruritic skin lesions on the scalp and limbs. These were thought to be atopic dermatitis, but considered atypical because of the distribution of the lesions involving flexor and extensor aspects of the limbs equally, and because of the intense exfoliation coupled with little or no lichenification. On this occasion multiple bruises and petechial haemorrhages were observed on the trunk and a platelet count of $2000/\text{mm}^3$ was recorded (Fig. 1). When examined he had chronic otitis media which had been active for 5 months preceding return to the dermatology clinic and from which antibiotic-resistant strains of *Staphylococcus aureus* and *Haemophilus influenzae* had been cultured.

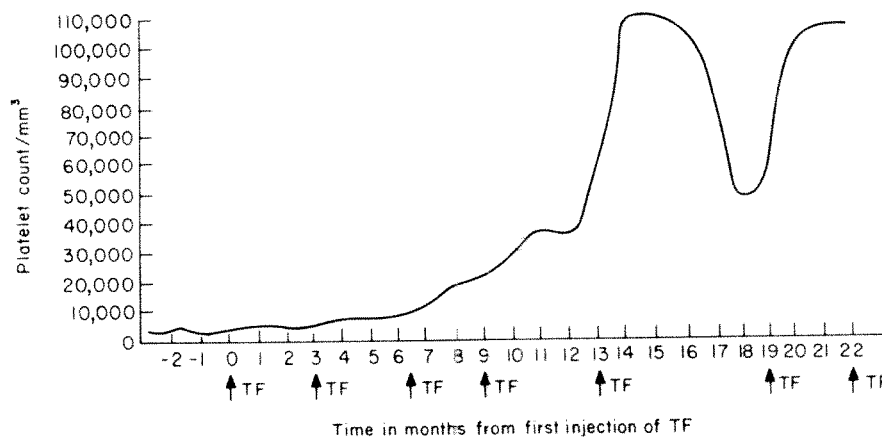


FIGURE 1. Changes in platelet level during transfer factor therapy. Each arrow indicates one unit of transfer factor administered subcutaneously.

Full general and immunological investigation was therefore instigated with the following results: Height and weight within 25th percentile. Spleen palpable, no hepatomegaly or lymphadenopathy. Hb 9.2 g/dl, WBC $2000/\text{mm}^3$, differential count showing eosinophilia (11%) and lymphopenia (26%).

Blood group A Rh. +ve with no detectable Anti B substance.

Bone marrow aspirate showed normal cellularity and normal numbers of megakaryocytes with no nuclear malformations.

Serum levels of IgG, A and M were all within the normal range and serum IgE was slightly elevated at 219 units/ml.

Table 1 shows these initial levels and charts the changing levels during therapy.

Individual complement components were assessed and were as follows (expressed as percentage of normal serum):

CH₅₀ level 960 u/ml Control 1600 u/ml.

C1 170%, C2 140%, C3 80%, C4 91%, C5 144%, C6 77%, C7 110%, Factor B 79%.

Cell mediated immune function assessment revealed cutaneous anergy by negative readings to skin testing with PPD, Candida DHS (Bencard), Mumps (Eli Lilly) and SKSD (Lederle) and also by failure to sensitize to 2000 µg of DNCB. Lymphocyte transformation in response to the plant mitogens phytohaemagglutinin, pokeweed mitogen and concanavalin A was within normal limits. Quantification of rosette forming lymphocytes in the peripheral blood revealed a very low level of E rosette forming cells (T lymphocytes) at 6% or $120/\text{mm}^3$, but a normal level of EAC rosette forming cells at 35% or $700/\text{mm}^3$.

Neutrophil chemotaxis to casein showed defective cell movement by comparison with an age-

TABLE 1. Changes in immunoglobulin levels during TF therapy

Normal range	IgG 550-1450 mg/100 ml	IgA 32-189 mg/100 ml	IgM 42-165 mg/100 ml	IgE under 200 units/ml
Prior to therapy	710	108	68	219
After 1 unit TF	1330	148	58	631
After 2 units TF	813	196	43	353
After 3 units TF	629	179	44	666
After 4 units TF	604	370	25	1650
After 5 units TF	547	258	23	1800
After 6 units TF	619	251	27	2450
Present levels	586	227	22	> 4000

matched control (patient 69 μ m in 75 mins, control 87 μ m). Similarly monocyte chemotaxis was defective (patient 51 μ m in 75 min, control 98 μ m).

In view of the clinical situation with a dangerously low platelet count and persistence of antibiotic resistant ear infection, and the immunological findings of cutaneous anergy, low T lymphocyte levels and defective chemotaxis, a therapeutic trial of transfer factor (TF) was considered justified. One unit of TF (the product of one pint of whole blood) was prepared by the method of Lawrence (1974) from a healthy donor who was skin test positive on testing to PPD. This was administered by subcutaneous injection, and as no *in vivo* or *in vitro* response was observed after one injection, two further units also from PPD positive donors were given on two separate occasions. After three units of TF had been given at 8 week intervals a rise in the platelet count was noted and this rise was accelerated after the 4th and 5th units of TF were given (Fig. 1). During this period the child's resistance to infection became noticeably improved and his antibiotic requirements which had been continuous for 7 months prior to TF therapy diminished significantly. However, during this period of improvement in haematological state and resistance to infection, a deterioration of the child's skin lesions was observed. When his platelets were above a level of 75,000/mm³ he was erythematous, and had pruritic exfoliating lesions on face and limbs.

As a spontaneous improvement in this condition could theoretically occur, TF was withheld for a period of 4 months to see if this influenced the child's condition. A sharp drop in platelet count to 60,000/mm³ and recurrence of otitis media occurred. After the administration of a 6th unit of TF the platelet count rose again, and the ear infection responded to antibiotic therapy. Since this time the child has been maintained on one unit of TF given every 8 weeks. At present he is on no antibiotic therapy, his platelet count is stable at 90,000-100,000/mm³, and his white count has risen to 8000-10,000/mm³. The otitis media is quiescent but he has continuous severe pruritic cutaneous lesions which do not respond to topical steroid applications.

Changes in immunological parameters after TF therapy have been a rise in circulating E rosette forming cells from 6% (120/mm³) to 40% (1040/mm³), the acquisition of a positive skin test to PPD, and the development of normal neutrophil and monocyte chemotaxis. No anti-platelet antibodies have developed and testing for auto-antibodies has been negative. The changes in immunoglobulin levels are charted in Table 1. The main features are a slight rise in IgA levels, a fall in IgM levels, and a steady rise in IgE from 219 units/ml to the present level of over 4000 units/ml. Identification of specific IgE by the RAST technique has shown it to be directed against egg white, dog epithelium and milk.

DISCUSSION

Although not an unduly rare disease in the world literature, cases of Wiskott-Aldrich syndrome appear to be relatively unusual in Britain as only three previously reported British cases appear in the literature (Gordon, 1960; Srivastava, 1967). The prognosis is uniformly poor, death usually occurring in the first decade from overwhelming infection or reticuloendothelial malignancy (ten Bensel, Stadlan & Krivit, 1966). The only treatment to date reported to be of benefit has been administration of transfer factor (Spitler, *et al.*, 1972). A series of 12 cases of Wiskott-Aldrich syndrome treated in this way showed benefit in the form of improved resistance to infection in approximately 50% of cases. In addition to this study, there are scattered reports of TF treatment of individual cases, but in no other study has such a clear cut relationship between TF administration and platelet count been demonstrated. Similarly there is no other account of an inverse relationship between the platelet count and severity of cutaneous involvement in this condition although if TF is acting as a stimulant of cell mediated immunity as is postulated, such an exacerbation could be considered a logical result of such therapy. The child is currently on a diet free of eggs and cow's milk, with as yet little change in his clinical state.

The postulated explanations for the triad of thrombocytopenia, dermatitis and recurrent infections in this condition range around a disorder of immunoprotein metabolism related to antigen recognition. It would appear from this case, however, that recognition of specific allergens as demonstrated by the RAST technique is certainly possible in these patients. An interesting biochemical study of three affected members of a family suggest that the common factor in haematological and immunological abnormalities is a granular defect in macrophages and platelets resulting in poor oxidative phosphorylation (Kuramoto, Steiner & Baldini, 1970). This could cause rapid platelet destruction due to physical stress, and loss of antigen capture and processing by macrophages.

If the cause of platelet destruction were indeed mechanical due to platelet fragility, then it would be necessary to postulate in this case that TF has partially corrected this abnormality. As Sandier has shown that one non-specific feature of TF activity is cyclic GMP stimulation, this may be the pathway by which this is achieved (Sandler *et al.*, 1975).

In vitro changes observed following TF therapy have included the rise in T lymphocytes and improved neutrophil and monocyte chemotaxis. In other immunodeficiencies rise in T cells after TF administration has previously been reported by Wybran *et al.* (1973), and correction of chemotaxis after TF therapy in a case of chronic mucocutaneous candidosis has been observed (Wilkinson, personal communication).

The most striking change, however, has been the steady and relentless rise in IgE levels from a level at the upper limit of normal prior to TF therapy to the present level of over 4000 units/ml. The dermatitis component of the Wiskott-Aldrich syndrome is generally considered to be atopic in type although several authors comment on the atypicality and tendency to erythroderma (Mills & Winkelmann, 1958; Kildeberg, 1961; Wolff, 1967). There does not appear to be any report in the literature of a child with this syndrome having either allergic asthma or rhinitis, and reports of a family history of atopy are similarly lacking. The IgE levels and finding of specific IgE in RAST testing in this case would lend support to the atopic nature of the skin lesions. The mechanism underlying excessive IgE production in atopy and the relationship of IgE to cutaneous lesions are at present poorly understood (Rajka, 1975). This would seem to be a fruitful field for studies of loss of suppressor T cell activity giving rise to uncontrolled IgE production.

The outlook for children with this condition appears to be uniformly poor, the majority dying before puberty from infection, haemorrhage or lymphoreticular malignancies (ten Bensel, Stadlan & Krivit, 1966). It is hoped however that immune manipulation will improve this prognosis in these children.

REFERENCES

- ALDRICH, R.A., STEINBERG, A.C. & CAMPBELL, D.C. (1954) Pedigree demonstrating a sex linked recessive condition characterised by draining ears, eczematoid dermatitis and bloody diarrhoea. *Paediatrics*, **13**, 133.
- TEN BENSEL, R.W., STADLAN, E.M. & KRIVIT, W. (1966) The development of malignancy in the course of the Aldrich syndrome. *Journal of Paediatrics*, **68**, 761.
- GORDON, R.R. (1960) Aldrich's syndrome. Familial thrombocytopenia with hypogammaglobulinaemia. *Archives of Diseases in Childhood*, **91**, 564.
- KILDEBERG, P. (1961) The Aldrich syndrome. Report of a case and discussion of pathogenesis. *Paediatrics*, **27**, 362.
- KURAMOTO, A., STEINER, M. & BALDINI, M.C. (1970) Lack of platelet response to stimulation in the Wiskott-Aldrich syndrome. *New England Journal of Medicine*, **282**, 475.
- LAWRENCE, H.S. (1974) Transfer factor in cellular immunity. *The Harvey Lectures Series* 68. Academic Press, New York.
- MILLS, S.D. & WINKELMANN, R.K. (1958) Eczema, thrombocytopenia, purpura and recurring infections. *AMA Archives of Dermatology*, **79**, 466.
- RAJKA, G. (1975) *Atopic Dermatitis*, pp. 73-77. Saunders, London.
- SANDLER, J.A., SMITH, T.K., MANGANELLO, V.C. & KIRKPATRICK, C.H. (1975) Stimulation of monocyte GMP by leukocyte dialysates. An antigen-independent property of transfer factor. *Journal of Clinical Investigation*, **56**, 1271.
- SPITLER, L., LEVIN, A.S., STITES, D.P., FUDENBERG, H.H., PIROFSKY, B., AUGUST, C.S., STIEHM, R., HITZIG, W.H. & GATTI, R.A. (1972) The Wiskott-Aldrich syndrome. Results of transfer factor therapy. *Journal of Clinical Investigation*, **51**, 3216.
- SRIVASTAVA, R.N. (1967) Wiskott-Aldrich syndrome. *Archives of Diseases in Childhood*, **42**, 604.
- WISKOTT, A. (1937) Familiarer Angeborenes Morbus Werlhofii. *Monatsschrift für Kinderheilkunde*, **68**, 212.
- WOLFF, J.A. (1967) Wiskott-Aldrich syndrome. Clinical, immunological and pathological observations. *Journal of Paediatrics*, **70**, 221.
- WYBRAN, J., LEVIN, A.S., SPITLER, L.E. & FUDENBERG, H.H. (1973) Rosette forming cells, immunologic deficiency diseases and transfer factor. *New England Journal of Medicine*, 710.

Familial lichen planus

ANOTHER DISEASE OR A DISTINCT PEOPLE?

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SUMMARY

When lichen planus strikes families, a very rare happening, it is likely to afflict younger members, to erupt more acutely, extensively, and gravely, attacking also nails and mucous membranes, and to recur.

Ten patients with familial lichen planus, two each from five distinct Caucasian families, the parents of whom were unrelated by birth, were found to be carrying HLA-B7 statistically more frequently than in the normal population or in those with the characteristic forms of lichen planus. We hint that their genotype might have rendered them susceptible to a pathogen that precipitated their disease.

Being an uncommon disease of mysterious origin, more than one patient with lichen planus (LP) in a household or a family invites epidemiological speculations (Hellier, 1950); or as in the report of Saffron (1940) in which four patients from one family developed LP, it might suggest a genetic diathesis rather than an infective origin as they all were living apart.

In our five families we found HLA-B7 more often than in our patients with the ordinary sporadic LP, so often a milder disease. This serologically demonstrated human histocompatibility lymphocytic antigen (HLA) in the course of evolution may have bestowed benefits, but would appear in this instance to confer some selective disadvantage upon the individual and imbue his skin with the propensity to erupt in this form. Maybe the precipitating factor is some unidentified agent, perhaps infective.

PATIENTS (TABLES 1 AND 2)

First family

Mrs R, aged 37 years. She developed LP at the age of 11. LP recurred when she was 20 years old whilst she was carrying SR. This, her third attack, started 2 years ago. The signs were widespread LP with small papules diffusely on trunk and arms, and hypertrophic lesions on the legs. Her nails were badly affected. Treatment with systemic corticosteroids was obligatory.

SR, her son aged 15 years. He has had LP for 9 months. Small papules were diffusely and profusely distributed on trunk and arms, and the lesions on the feet were bullous. Mouth and nails were affected. The eruption was controlled only after systemic corticosteroids.

TABLE 1. LP: Findings in the 6 affected members of the 3 families studied

Patient	Age of onset and recurrences	Sites of lesions				Systemic steroids	HLA			
		limbs, trunk, mouth, nails					locus A		locus B	
R, mother	11, 20, 35	+	+	—	+	Yes	1	3	7	W10
SR, son	14	+	+	—	+	Yes	1	10	7	8
AP, brother	10, 14	+	+	—	—	Yes	1	—	7	13
NP, brother	6	+	—	+	+	No	1	—	7	12
M, mother	54	+	+	+	+	No	3	11	7	14
M, daughter	23, 24	+	+	—	+	Yes	2	11	7	14

TABLE 2. LP: the two families studied by Drs Woodrow and Lowe

Family A	HLA	
	Locus A	Locus B
M.D. Mother	2 3	12 14
H.D. Daughter	3 9	12 14
Family B	HLA	
	Locus A	Locus B
P Mother	1 3	7 8
E.D. Son	3 —	7 W10

Second family

AP, aged 14 years. He developed widespread LP at the age of 10 years. Systemic steroids were essential to control the eruption. His second severe attack started 6 months ago when the LP was widespread with small papules diffusely over the trunk and arms, and hypertrophic lesions on legs.

NP, his brother aged 6 years. LP had erupted 6 months earlier with annular and hypertrophic lesions on legs and a profuse eruption on trunk and arms. Mouth and nails were affected.

Third family

Mrs M, aged 54 years. Myriads of small papules had been distributed diffusely over trunk and arms for 4 months. Mouth and nails were affected.

Miss M, her daughter aged 24. She had developed LP for the first time aged 23, and the second attack had started 2 weeks before she presented. Small papules in vast numbers had erupted on the trunk and arms. Her nails were affected. We treated her with systemic corticosteroids.

Fourth and fifth families

(Lowe, Cudworth & Woodrow, 1976)—Table 2.

Ordinary sporadic classical lichen planus

Thirty patients were studied at the same time.

METHOD

The HLA antigens were identified using a standard modified two-stage micro-lymphocytotoxicity method (Terasaki & McClelland, 1964) on lymphocytes separated from heparinized blood using the Böyum (1968) density gradient method and tested for 22 HLA antigens using a panel of 60 different typing sera. Each antigen was typed according to the properties and performance of the sera during the course of the complete investigation, their assignment being decided upon from two or three typing sera in most cases.

The 'control population' comprised 600 Caucasians who were being tested by the Tissue Typing Laboratory, Westminster Hospital, while our study on the LP patients was in progress during the search for marrow transplant donors.

RESULTS

We have studied three families ('3F' group, Table 1) with lichen planus, in each of which two members were affected, and we were fortunate to add those of the two other families (Table 2) from Dr Woodrow (Lowe, Cudworth & Woodrow, 1976) so that the familial LP group ('5F' group) numbered 10 patients.

The HLA serological determinants of the familial LP patients ('5F' group) were compared using the Fisher exact probability test with the 'control population' of 600 normal subjects (Control group), and the 30 sporadic and non-familial LP patients ('Non-F' group) using the same sera.

Related people do not represent statistically 'independent events', because of their inherent association which will favour the transmission of significantly similar proportions of HLA groupings. To overcome this difficulty only one member of each independent family with familial LP was included in the sample for comparison. This raised the problem of which member to include since it would be possible for different combinations to give different results. In practice, all combinations of individual family members were examined; the quoted probabilities representing the mean of these results. All probabilities are two-tailed. The conclusions were:

- (1) The '5F' group differs from the 'Control' group:
HLA-A3 occurs more often in the '5F' group $P=0.037$.
HLA-B7 occurs more often in the '5F' group $P=0.009$.
- (2) The '5F' group differs from the 'Non-F' group:
HLA-A3 occurs more often in the '5F' group $P=0.035$.
HLA-B7 occurs more often in the '5F' group $P=0.003$.

The probabilities for HLA-B7 were independent of which members were chosen from each family.

DISCUSSION

We have found the HLA-B7 serological determinant in proportionally more patients with the rarely encountered familial LP than in those with typical patterns of LP, or in the general population (control) group. Familial LP would seem to be a more severe disease than ordinary LP with an earlier age of onset, extensive lesions, and the likelihood of recurrences.

The very rarity of familial cases of lichen planus has long tempted inquiries about infective agents (Saffron, 1940; Hellier, 1950), but only a few (Swanbeck & Thyresson, 1964; Brody, 1965) claim to have found microorganisms in LP lesions. The disease, nevertheless, could be a reaction pattern precipitated by infective agents, the agent having long since disappeared by the time the eruption has developed. Lowe, Cudworth & Woodrow (1976) suggested that if others were to confirm their suspicions of an association of LP with the HLA system, this would point to some HLA gene-linked susceptibility. We were invited to add their data on two families to those on our three, because a family study could be expected to emphasize a linkage between the disease susceptibility gene and the HLA system where it is not an over strong one.

LP may be an immunological disease as is suggested by the discovery of immune reactants in the lesions (Copeman, 1970), the association with diseases characterized by organ specific antibodies (Tan, 1973a and b), and its occasional merging with lupus erythematosus (Copeman, Schroeter & Kierland, 1970). The destruction of the epidermal basal layer in LP accounts for the narrow distinction between bullous LP and LP pemphigoides (Sobel, Miller & Shatin, 1976), and appears to predispose the exceptional individual to develop humoral antibodies against the basement membrane zone so that the staining artefacts of an apparently true pemphigoid are provoked (Stingl & Holbar, 1975; Rekauf, 1976).

The dermal mononuclear infiltrate in LP was found to be composed largely of T lymphocytes (Tan, Byrom & Hayes, 1975), the surface markers of which are unaffected (Tan, 1976) by the use of collagenase in the disaggregation process. These T lymphocytes may be reacting to the destruction some agent has wrought in the basement membrane zone and be contributing to the coating of the epidermal cells with immune reactants transforming them into hyaline bodies (Ueki, 1969). Alternatively T lymphocytes, independently of the antibody and complement systems, through their lymphokines, may chemotactically attract and activate phagocytes, neutrophils and mononuclears (David, 1975).

The signs in LP and in lupus erythematosus may be difficult to distinguish and in each disease be so variable that the overlap might be blamed on a common aetiological agent (Davies *et al.*, 1977), the pathological consequences of which are being expressed in different genetic backgrounds. The genotypic associations are unproven, but seemingly systemic lupus erythematosus is allied to HLA-B7 (Millard, Rowell & Rajah, 1977). Information derived in the future from the lymphocyte rather than the serological determinants of the HLA system, for example, might emphasize or refute that patients with acute familial LP are genetically special people, or contrarily signify that LP is not a single entity.

CONCLUSION

Some of the many reports of disease associations with the HLA-system have been blamed upon the patient's predisposition to (virus) infection governed by certain genes segregating at one or just a few loci. For example, defective T-cell immunity is blamed for the natural clustering of HLA-A3 and B7 genes in Caucasians (Bach & van Rood, 1976) notably in the cases of multiple sclerosis (*Lancet*, 1976) and poliomyelitis (Morris & Pietsch, 1973). The HLA antigens, genetic markers of the individual's biological uniqueness, are intimately connected with the determination of immune responses (Widmer & Bach, 1974; Tauber, 1976; Munro & Bright, 1976), whatever the trigger. From our small sample of patients with the rare and severe form of LP as is seen in the familial cases, we are tempted to question whether their genetic setting increases their vulnerability and probable immunogenetic response to an external influence. The culprit, perhaps one commonly encountered in the environment, could be a chemical contaminant, food additive, drug, native medicine, infective agent, or a derived product or metabolite. The response in most will not be manifested as a rash and thus will pass unrecognized as LP. A parallel can be found in poliomyelitis, which is non-paralytic in most patients, but in those

whom it paralyses, say 1%, an HLA-A3 and B7 genetic background is found. But then familial LP may be a different disease, distinct from the more indolent eruptions normally diagnosed as LP. Whether familial LP signifies a different disease or a particularly susceptible population, it is a disabling malady needing powerful remedies.

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REFERENCES

- BACH, F.H. & VAN ROOD, J.J. (1976) The major histocompatibility complex—genetics and biology. *New England Journal of Medicine*, **295**, 806.
- BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at Ig. *Scandinavian Journal of Clinical Laboratory Investigation*, **21**, Suppl. **97**, 77.
- BRODY, I. (1965) Electron-microscope demonstration of bacteria in the skin of patients with lichen planus. *Nature (London)*, **207**, 96.
- COPEMAN, P.W.M. (1970) Investigations into acute cutaneous angitis. *British Journal of Dermatology*, **82**, Suppl. **5**, 51.
- COPEMAN, P.W.M., SCHROETER, A.L. & KIERLAND, R.R. (1970) An unusual variant of lupus erythematosus or lichen planus. *British Journal of Dermatology*, **83**, 269.
- DAVID, J.R. (1975) A brief review of macrophage activation by lymphocyte mediators. In: *The Phagocytic Cell in Host Resistance* (Ed. by J.A. Bellanti and D.H. Dayton), pp. 143–153. Raven Press, New York.
- DAVIES, M.G., GORKIEWICZ, A., KNIGHT, A. & MARKS, R. (1977) Is there a relationship between lupus erythematosus and lichen planus? *British Journal of Dermatology*, **96**, 145.
- HELLIER, F.F. (1950) Familial lichen planus. Is it significant evidence of infectivity? *British Journal of Dermatology*, **62**, 446.
- LANCET: leading article (1976) Histocompatibility antigens and multiple sclerosis. *Lancet*, **ii**, 1286.
- LOWE, N.J., CUDWORTH, A.G. & WOODROW, J.C. (1976) HL-A antigens in lichen planus. *British Journal of Dermatology*, **95**, 169.
- MILLARD, L.G., ROWELL, N.R. & RAJAH, S.M. (1977) Histocompatibility antigens in discoid and systemic lupus erythematosus. *British Journal of Dermatology*, **96**, 139.
- MORRIS, P.J. & PIETSCH, M. (1973) A possible association between paralytic poliomyelitis and multiple sclerosis. *Lancet*, **ii**, 847.
- MUNRO, A. & BRIGHT, S. (1976) Products of the major histocompatibility complex and their relationship to the immune response. *Nature (London)*, **264**, 145.
- SAFFRON, M.H. (1940) Familial lichen planus—a report of four cases of lichen planus in one family. *Archives of Dermatology and Syphilology*, **42**, 653.
- SOBEL, S., MILLER, R. & SHATIN, H. (1976) Lichen planus pemphigoides: immunofluorescence findings. *Archives of Dermatology*, **112**, 1281.
- SWANBECK, G. & THYRESSON, N. (1964) Electron microscopy of intranuclear particles in lichen ruber planus: a preliminary report. *Acta dermato-venereologica*, **44**, 105.
- TAN, R.S.-H. (1973a) Thymoma, acquired hypogammaglobulinaemia, lichen planus, alopecia areata. *Proceedings of the Royal Society of Medicine*, **67**, 196.
- TAN, R.S.-H. (1973b) Ulcerative colitis, myasthenia gravis, atypical lichen planus, alopecia areata, vitiligo. *Proceedings of the Royal Society of Medicine*, **67**, 195.
- TAN, R.S.-H., BYROM, N.A. & HAYES, J.P. (1975) A method of liberating living cells from the dermal infiltrate. Studies on Skin Reticulosis and LP. *British Journal of Dermatology*, **93**, 271.
- TAN, T. (1976) The effect of collagenase on surface markers of human peripheral lymphocytes. *British Journal of Dermatology*, **95**, 361.
- TAUBER, J.W. (1976) 'Self': Standard of comparison for immunological recognition of foreignness. *Lancet*, **ii**, 291.
- UEKI, H. (1969) Hyaline bodies in subepidermal papillae. *Archives of Dermatology*, **100**, 610.
- WIDMER, M.B. & BACH, F.H. (1974) *Federation Proceedings*, **33**, 720.

Familial lichen planus

A CASE REPORT

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SUMMARY

A Jewish Shirazee family is described in which 6 members had lichen planus. There was no increased incidence of the phenotype HLA A3.

Familial lichen planus is not common. When Hellier (1950) reviewed the literature he was able to find reports of only 51 cases and Samman (1961), in a survey of 200 consecutive cases, found only three. In most familial reports only 2 people have had the disease but Saffron (1940) described a family of 7 in whom the mother and 3 of 4 sons had the disease and referred to 4 families in which 3 people were affected. Goldstein (1942) also described a family in which a mother and her son and daughter were affected.

Recently we were able to examine a Jewish family in Shiraz (Fig. 1) in which a mother and 5 of her

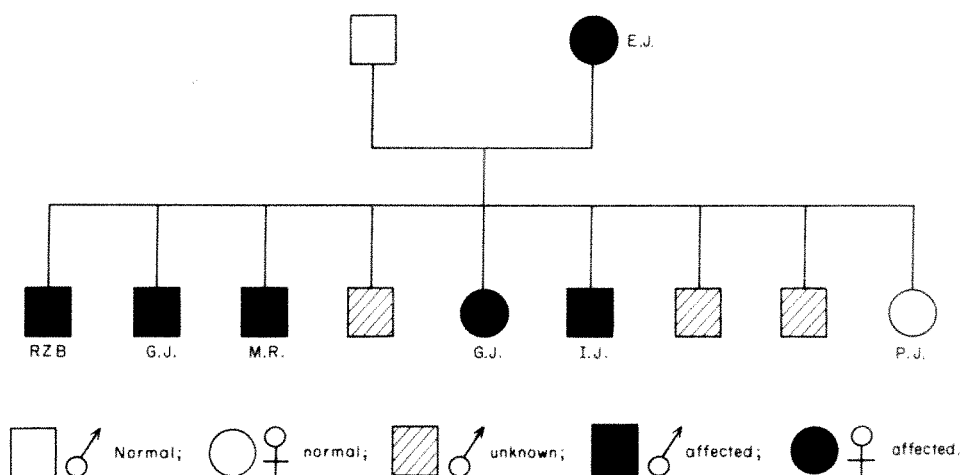


FIGURE 1. Pedigree of Shirazian family with LP.

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9 children had the typical skin lesions of lichen planus. Five of them had classical hypertrophic lichen planus on the shins; one of them had recently developed generalized lichen planus and the sixth had generalized lichen planus with no preceding hypertrophic plaques. None of them had mucosal involvement. One daughter was clear and 3 sons, who were not examined as they lived abroad, were said to have a skin disease. The duration of the disease varied from 8 months to 15 years and all the members had developed skin lesions at different times. The clinical diagnosis was obvious but in 3 patients it was confirmed histologically. The hypertrophic lesion showed hyperkeratosis, hypergranulosis, irregular acanthosis, liquefaction of the basal cell layer and a band like monocyctic infiltrate in the dermal papillae and upper dermis. There was no evidence of amyloid. HLA typing was done in 5 affected and 1 unaffected members of the family using a microcytotoxicity test with specially prepared antisera for locus A and B (Table 1).

TABLE 1. Clinical details of LP and HLA phenotypes in 7 members of the family

Age	Clinical picture	Duration (years)	HLA phenotypes
65	EJ Hypertrophic on shins	2	HLA Aw33 A29 HLA B13 B18
50	RZB Hypertrophic on shins	10-15	HLA Aw33 A29 HLA B8 B14
48	GJ Hypertrophic on shins + generalized	10-15 1/12	Not done
46	MR Hypertrophic on shins	10	HLA A1 Aw33 HLA B12 B13
40	IJ Hypertrophic on shins	10	HLA A9 HLA B14 Bw35
40	GJ Generalized	8/12	HLA A3 HLA B12 B14
29	PJ No disease		HLA A9 Aw24 HLA B14

DISCUSSION

This family in which a mother and 5 of her 9 children were seen to have active lichen planus seems to be the largest familial group in the literature. It was disappointing that 3 other sons who had a skin disease could not be examined.

Although Erasmus Wilson (1869) described classical lichen planus more than a century ago, the aetiology is still unknown. Autoimmunity has been postulated (Sarkany, 1972; Lachapelle & De la Brassine, 1973), and also an increased susceptibility to an unknown virus as a result of deficiency in circulating IgA and IgM (Stankler, 1975). Recently, however, in a study of 57 patients with lichen planus a significant increase in the frequency of human leukocyte antigen A3 was shown (54% compared with 29.7% in a control population) and it was suggested that there was a gene on the HLA chromosomal region causing increased susceptibility to the disease (Lowe, Cudworth & Woodrow, 1976).

In our family, all the members developed the disease at widely spaced intervals in time so that an infective cause seemed unlikely. In view of Lowe *et al.*'s findings, leukocyte antigen studies were performed in 6 members of the family to see if they had a significant increase in HLA A3. In the 5 affected members of this family who were examined, HLA B14 was found in three. HLA Aw33, A9

and B13 were found in 2 members and HLA A3 was present in one member (Table 2). The disease-free member had the phenotype HLA-A29 Aw33 B14 B12. The relative frequencies of the mentioned phenotypes in the control population of 100 consecutive Shirazee blood donors are shown in Table 2. HLA B14 was found in only 5% of the control population; HLA A3 was present in 11% of controls. Although HLA B14 was found in 3 affected members in this family, it seems unlikely that it is linked to a tendency to develop lichen planus as one affected son did not have it and one unaffected daughter did. It was unfortunate that only 6 of the family could be typed. One can only say that in this particular family the predisposition to LP was not linked to HLA A3 as it was in 54% of Lowe *et al.*'s patients.

TABLE 2. Incidence of HLA Phenotypes in the LP patients and the control population

	LP patients (n=5)	Controls (n=100)
A Locus		
HLA Aw33	2	22
HLA A 29	1	9
HLA A 9	2	23
HLA A 3	1	11
HLA A 1	1	21
B Locus		
HLA-B12	1	15
HLA-B13	2	7
HLA-B14	3	5
HLA-B18	1	14
HLA-Bw35	1	34

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REFERENCES

- GOLDSTEIN, M.B. (1942) Familial lichen planus. *Archives of Dermatology and Syphilology*, **45**, 1166.
 HELLIER, F.F. (1950) Familial lichen planus. Is it significant evidence of infectivity? *British Journal of Dermatology*, **62**, 446.
 LACHAPELLE, J.-M. & DE LA BRASSINE, M. (1973) The proliferation of cells in the dermal infiltrate of lichen planus lesions. *British Journal of Dermatology*, **89**, 137.
 LOWE, N.J., CUDWORTH, A.G. & WOODROW, J.C. (1976) HLA antigens in lichen planus. *British Journal of Dermatology*, **95**, 169.
 SAFFRON, M.H. (1940) Familial lichen planus. *Archives of Dermatology and Syphilology*, **42**, 653.
 SAMMAN, P. (1961) Lichen planus. An analysis of 200 cases. *Transactions of the St John's Hospital Dermatological Society*, **46**, 36.
 SARKANY, I. & GAYLARDE, P.M. (1972) The pathogenesis of lichen planus. *British Journal of Dermatology*, **87**, 81.
 STANKLER, L. (1975) Deficiency of circulating IgA and IgM in adult patients with lichen planus. *British Journal of Dermatology*, **93**, 25.
 WILSON, E. (1869) On leichen planus. *Journal of Cutaneous Medicine*, **3**, 117.

Brief Communications

Complement activation by *Corynebacterium acnes*

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Corynebacterium (Propionibacterium) acnes is described as the causative organism in acne vulgaris, though its precise role has yet to be determined. It is present as a member of the normal flora of the facial skin of all persons after puberty, the numbers increasing steadily from puberty to 35–40 years. Patients with severe acne have significantly higher levels of complement fixing antibodies to *C. acnes* than do those with mild acne or no acne (Puhvel, Hoffman & Sternberg, 1966). Skin biopsies of acne lesions show deposition of complement component C3 (Dahl & McGibbon, 1976).

Commins & Johnson (1974) reported *C. parvum* to be a synonym of *C. acnes*. *C. parvum* has been shown to consume C3 in human serum in which the antibody-mediated classical pathway is inhibited (McBride *et al.*, 1975). In view of this, we decided to test *C. acnes* in a system for detecting activation of the alternative pathway by the cleavage of C3 in normal human serum. Samples of serum were incubated with *C. acnes* at 37°C for 30 min. in the presence of 5mM MgEGTA to inhibit classical pathway complement activation. Breakdown of C3 to C3b was measured by Laurell two-way electrophoresis in agarose gel containing anti-human C3. Normal serum, and serum deficient in C2, supported conversion of C3 to C3b by *C. acnes*, whereas a prepared serum reagent with classical pathway mediated haemolytic activity of 67% but deficient in Factor D of the alternative pathway did not. We have screened a large number of other substances and microorganisms in this way and have found that C3 breakdown can be mediated by gluten fractions (Massey, Capner & Mowbray, 1977) and by many strains of *M. Lepraemurium* and preparations of its mycoside fraction (Wax D) (unpublished results).

These agents which we report to activate the alternative pathway share to some extent the characteristics of adjuvants, mitogens and activators of macrophages. An important finding is that C3b itself is able to activate macrophages (Schlorlemmer & Allison, 1976), and so perhaps the generation of C3b via the alternative pathway is an early step in these mechanisms.

It is a long step from such speculation on laboratory results to an explanation of the pathogenesis of a disease. However, we can now group together some diseases where the causative agent can cleave C3, and where the disease manifests inflammation. Following this pattern, *C. acnes* in acne can be included with gluten in dermatitis herpetiformis (DH), *Actinomyces viscosus* in periodontal disease (Allison, Schlorlemmer & Bitter-Suerman, 1976), and probably some mycobacteria in their associated skin conditions.

It seems possible that the presence locally of *C. acnes* in the upper layers of the skin may lead to local activation of the alternative complement pathway, with the local generation of C3b leading to chemotaxis and local lysosomal enzyme release. This latter would then represent the final inflammatory event in acne; other factors such as cellular immunity to the organisms may also play a role in production of the inflammatory lesion (Puhvel, 1977). Both mechanisms would be ablated by the reduction in organism counts produced by tetracycline, but by analogy with activity in diseases such

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as dermatitis herpetiformis, dapsone might find a place in treatment of the complement mediated pathway, although it would perhaps be less likely to affect lesions produced by cellular immunity.

REFERENCES

- ALLISON, A.C., SCHLORLEMMER, H.U. & BITTER-SUERMAN, D. (1976) Activation of complement by the alternative pathway as a factor in the pathogenesis of periodontal disease. *Lancet*, **ii**, 1001.
- CUMMINS, C.S. & JOHNSON, J.L. (1974) *Corynebacterium parvum*: a synonym for *Propionibacterium acnes*. *Journal of General Microbiology*, **80**, 433.
- DAHL, M.G. & MCGIBBON, D.H. (1976) Complement in inflammatory acne vulgaris. *British Medical Journal*, **iv**, 1383.
- MASSEY, A., CAPNER, P.M. & MOWBRAY, J.F. (1977) Activation of the alternate pathway by gluten: a possible aetiological factor in dermatitis herpetiformis. *Immunology*, **33**, 339.
- MCBRIDE, W.H., WEIR, D.M., KAY, A.B., PEARCE, D. & CALDWELL, J.R. (1975) Activation of the classical and alternate pathways of complement by *Corynebacterium parvum*. *Clinical and Experimental Immunology*, **19**, 143.
- PUHVEL, S.M., HOFFMAN, I.K. & STERNBERG, T.H. (1966) Levels of complement-fixing antibodies to *Corynebacterium acnes* in sera of patients with acne vulgaris. *Archives of Dermatology*, **93**, 364.
- PUHVEL, S.M., AMIRIAN, D., WEINTRAUB, J. & REISNER, R.M. (1977) Lymphocyte transformation in subjects with nodulo-cystic acne. *British Journal of Dermatology*, **97**, 205.
- SCHLORLEMMER, H.U. & ALLISON, A.C. (1976) Effects of activated complement components on enzyme secretion by macrophages. *Immunology*, **31**, 781.

People without navels

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Epidermolysis bullosa dystrophica-recessive is a rare scarring dermolytic bullous dermatosis. A minor sign of this disease, not previously mentioned, is absence of the umbilicus. Over the past 7 years I have had occasion to examine 2 such patients who had no history of surgical or other external trauma to the mid-belly. One patient, in her twenties, and the other, in his teens, had long ago suffered the replacement of the umbilicus by a flat expanse of scar. It is surmised that in this disease inevitable trauma to the natal navel leads to early blisters, obliteration and scar. The seemingly unique absence of umbilicus may constitute a diagnostic sign of congenital scarring dermolytic disease.

Comment

NEW CONCEPTS IN THE PATHOGENESIS OF PSORIASIS

The pathogenesis of psoriasis has stimulated many studies. We suggested in 1972 that immunological factors could explain the biochemical abnormalities of the psoriatic lesion (Rimbaud *et al.*, 1972b) and subsequently at the 1975 French National Dermatological Society Meeting we proposed an immunobiochemical theory (Guilhou & Meynadier, 1976).

In the light of numerous recent immunological, biochemical and ultrastructural investigations we can try to integrate all these findings, going step by step from the main epidermal feature (cellular proliferation) to the initial events of the pathogenesis of psoriasis (Fig. 1).

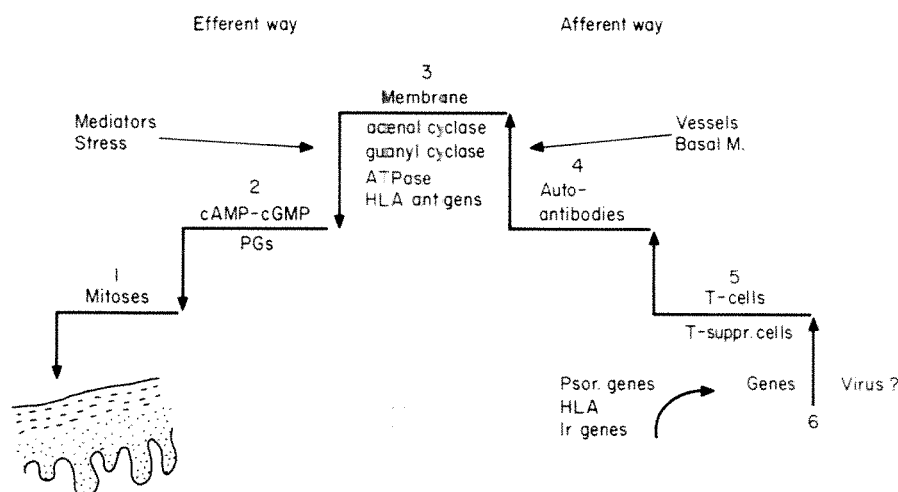


FIGURE 1. The six steps of the pathogenic chain of psoriasis.

The first 3 steps are well known: they are (1) epidermal proliferation, (2) cyclic nucleotide disequilibrium, (3) membrane abnormalities. They constitute the efferent way, leading from the membrane abnormalities to the basal histopathological lesion.

The steps of the afferent way, resulting in these membrane alterations, are more difficult to reconstitute and include immunological factors.

FIRST STEP: EPIDERMOPOIESIS

Psoriasis is an epidermal proliferative disease

Psoriatic epidermis was noted to have an extremely high number of mitoses (Van Scott & Ekel, 1963) and a rapid turn-over as demonstrated by isotopic techniques (Rothberg, Crounse & Lee, 1961). This proliferation results in a shortened transit time through the epidermis (Weinstein & Van Scott, 1965) and is associated with parakeratosis.

Is this epidermal proliferation due to a reduced cell division cycle time? (Weinstein & Frost, 1968). This point is controversial (Goodwin, Hamilton & Fry, 1974; Wright, 1976) but it is possible that proliferation is mainly brought about by a non-cycling cell recruitment (Gelfant, 1976).

SECOND STEP: CYCLIC NUCLEOTIDES — PROSTAGLANDINS

Increased epidermal cell proliferation can be explained by cyclic nucleotide and prostaglandin (PGs) imbalance

Epidermal cell renewal is regulated locally by epidermal chalones (Bullough, 1962) which inhibit the epidermal cell cycle in the G1 or G2 phase (G1 and G2 inhibitors). These inhibitors are catecholamine-dependent (Bullough & Laurence, 1964) and cyclic nucleotides are involved in their epidermal growth regulation, mainly for the G2 inhibitor (Voorhees & Duell, 1971; Elgjo, 1975).

The cyclic AMP/cyclic GMP ratio is most important in determining the proliferation and differentiation of cells (Voorhees, Marcelo & Duell, 1975).

Voorhees *et al.* (1972) reported that low cyclic AMP and high cyclic GMP levels (Voorhees *et al.*, 1973) account for cell proliferation, incomplete differentiation and glycogen accumulation in the psoriatic lesion. However, these data were not confirmed by two further studies (Harkonen, Hopsu-Havu & Raij, 1974; Yoshikawa *et al.*, 1975b) which found normal or high cyclic AMP levels in the lesions. It is likely that increased epidermal proliferation could be induced by an increase of cyclic GMP and subsequently by an increase of cyclic GMP/cyclic AMP ratio (Halprin, 1976; Voorhees *et al.*, 1976).

Furthermore *Adenyl Cyclase* (A.C.) activity has been demonstrated to be abnormal in the psoriatic lesion (Wright *et al.*, 1973; Harkonen *et al.*, 1974; Mei Mei Mui, Hsia & Halprin, 1975; Yoshikawa *et al.*, 1975a). This enzyme is poorly responsive to adrenaline stimulation (Wright *et al.*, 1973) but more responsive to PGE₂ (Mei Mei Mui *et al.*, 1975). These data suggest that the poor response in the involved epidermis is due to a reduced activity of membrane β adrenergic receptors (Yoshikawa *et al.*, 1975a).

Prostaglandins are important molecules in the modulation of cyclic nucleotide metabolism. Hammarstrom *et al.* (1975) demonstrated increased levels of free arachidonic acid, Hete (hydroxy-eicosatetraenoic acid), PGE₂ and PGF₂ in psoriatic lesions and an elevated PGF₂/PGE₂ ratio. But the PGE₂ synthesis from arachidonic acid could be reduced in psoriatic epidermis (Aso *et al.*, 1975); this could be due to the possible presence of a PG synthesis inhibitor (Penneys *et al.*, 1975).

The difficult problems of cyclic nucleotides and PGs in psoriasis are far from clear and need further studies. However, it is likely that the lack of control of the proliferation mechanism in psoriasis is the result of an imbalance affecting the cyclic AMP/cyclic GMP, adenyl cyclase/guanyl cyclase, PGE/PGF ratios.

THIRD STEP: THE PLASMA MEMBRANE

Abnormal growth control mechanism can be due to plasma membrane abnormalities

Cyclic nucleotide metabolism is dependent on membrane-bound enzymes (adenyl cyclase, guanyl cyclase) and on PGs which are mainly synthesized in the cell membrane.

Functional abnormalities of membrane-bound enzymes in psoriasis are not limited to the disturbance of adenyl cyclase (Wright *et al.*, 1973) but include a decrease of ATPase and acid phosphoesterase activities (Mahrle & Orfanos, 1974; Mahrle & Orfanos, 1975).

Numerous structural alterations of membrane components and of intercellular material have been noted in psoriatic epidermis: microvilli, pore-like structures on the cell surface (Orfanos, 1971;

Barrière, Litoux & Geraut, 1974), poorly developed tonofilaments and widened intercellular spaces (Brody, 1962), nearly complete absence of glycoprotein-rich surface coats (Orfanos *et al.*, 1973), and increase of gap junctions (Caputo, Pelucchetti & Innocenti, 1977).

Disturbance of histocompatibility (HLA) antigens (Russel, Schultes & Kuban, 1972; White *et al.*, 1972; Rimbaud *et al.*, 1974) located on cell surfaces could interfere with membrane hormonal receptors by different physical or biochemical mechanisms (Svejgaard & Ryder, 1976). In this way, they could modify the membrane receptor activities and disturb intracellular metabolism.

As underlined by Marilhe and Orfanos (1977) these numerous membrane abnormalities may explain the abnormal growth control of psoriatic epithelium. The cell membrane is the 'hinge point' of the pathogenic mechanisms in psoriasis. The membrane defect leads to the cell proliferation by the three steps of the efferent way described above.

Let us now consider the afferent way, which results in the membrane dysfunction.

FOURTH STEP: LOCAL IMMUNOLOGICAL PHENOMENA

Plasma membrane abnormalities could result from immunological processes located in psoriatic epidermis

Immunological data in psoriasis are relatively recent. Immunoglobulin disorders and a dissociated impairment of thymus-dependent lymphocytes have been reported (Guilhou *et al.*, 1976a; Guilhou *et al.*, 1976b).

Immunological phenomena in psoriatic epidermis can be summarized as: (1) presence of immunoglobulins and complement, (2) anti-IgG factors, (3) anti-stratum corneum antibodies, (4) anti-nuclear antibodies.

(1) Immunoglobulins and C3 fragment of complement have been found by Krogh & Tonder (1972) in psoriatic epidermis using a mixed agglutination technique.

(2) An anti-IgG activity consistent with rheumatoid factor of various Ig classes was also demonstrated in the lesions by the same authors (Krogh *et al.*, 1972; Krogh & Tonder, 1973). Moreover, such rheumatoid factors are found in psoriatic sera by an absorption-elution method (Howell *et al.*, 1972; Florin-Christensen *et al.*, 1974) or by indirect immunofluorescence techniques (Guilhou, Maynadier & Clot, 1973a). They are mainly IgG anti-IgG factors, not detected by classical agglutination techniques. Finally, our group (Rimbaud *et al.*, 1973) by using the rheumatoid rosette test, has demonstrated an anti-IgG activity on peripheral blood lymphocytes in psoriasis. It can be hypothesized that these anti-IgG antibodies, secreted by lymphocytes, could pass from the serum into the epidermis and be bound to antigen-antibody complexes.

(3) Anti-stratum corneum antibodies (SCAb) bound to the horny layer were also found by Krogh (1970). Beutner *et al.* (1975) and Jablonska *et al.* (1975) demonstrated their intercellular fixation by an immunofluorescence technique. These anti SCAB, normally present in the sera of healthy subjects (Krogh, 1970), do not usually have access to their homologous antigen, whereas they could pass through the dermo-epidermal barrier and form immune complexes in psoriatic epidermis.

(4) Finally, Cormane, Hunyadi & Hamerlink (1976) recently demonstrated the occurrence of anti-nuclear antibodies in parakeratotic nuclei of psoriatic lesions. These antibodies belonging to various classes of immunoglobulins are not detected in sera but on polymorphonuclear cells and lymphoid cells of the dermal infiltrate and of Munro-Sabouraud micro-abscesses. They could initiate a self-perpetuating antigen-antibody epidermal reaction, which would possibly be modified by PUVA therapy (Cormane *et al.*, 1977).

Analysis of these four points shows that complex immunological phenomena occur in psoriatic epidermis. The interaction of autoantibodies (anti-IgG, anti-SC, antinuclear) with their epidermal

antigen to form immune complexes initiates the complement fixation which leads to the generation of chemotactic factors and proteolytic enzymes. These different factors could account for the structural and functional membrane abnormalities of psoriatic keratinocytes.

FIFTH STEP: DEFECT OF CELLULAR ASPECTS OF THE IMMUNE RESPONSE

The disturbances of humoral immunity (auto-antibody production) could be related to an impairment of the control of the immune response by thymus-dependent lymphocytes

Such a control defect was suspected from the occurrence of auto-antibodies and from the increase of serum IgA levels (Fraser, Dick & Grichton, 1969; Marghescu & Braun-Falco, 1970; Varelzidis & Theodoris, 1971; Guilhou *et al.*, 1976a), salivary IgA (Oon *et al.*, 1973; Guilhou *et al.*, 1973a) and serum IgE (Guilhou *et al.*, 1976a). The IgA and IgE production is effectively thymus-dependent in mice as well as in man (Geha *et al.*, 1975).

We first demonstrated a T cell deficiency in a small group of patients (Clot *et al.*, 1975). These data were confirmed in a recent study of 60 psoriatic subjects (Clot *et al.*, 1977). A significant decrease of T cell numbers was shown by the diminution of three T-cell markers (E-rosettes, active E-rosettes and anti-human-T lymphocyte antigen serum). This defect seems to be correlated, in our experiments, to the extent of cutaneous involvement of the disease.

Furthermore, the lymphocyte response to concanavalin A stimulation was low in the psoriatic group, whereas phytohaemagglutinin and pokeweed mitogen induced normal responses. Such a dissociation could be in favour of a T-cell subset defect. If the situation in man is analogous to that in mice (Stobo & Paul, 1973), then our findings may identify a selective deficiency of the suppressor population. But direct evidence is far from clear at the present time.

Finally, we described a significant increase of serum thymic factor according to the patients' age. The discrepancies between high thymic hormone levels and low T cell numbers raise many hypotheses but remain unexplained. It is of interest to note the relationship reported between thymic factors, cyclic AMP and prostaglandins (Bach & Bach, 1973).

Cormane *et al.* (1976) and Glinski *et al.* (1977), investigating T cells by E-rosette tests, have reported a similar T-cell deficiency in psoriasis. However, other authors, studying small groups of patient with the E-rosette test, have reported conflicting data (Heid *et al.*, 1976; Levantine & Brostoff, 1975; Lischka, 1976). Such discrepancies could be linked to different stages of cutaneous lesions, since Glinski *et al.* (1977) have found a transient decrease of E-rosettes related to the activity of skin lesions.

Further studies are now needed to investigate T lymphocyte subpopulations in psoriasis and to elucidate a possible role of T-suppressor-cells in the autoimmune aspects of the disease.

SIXTH STEP: HYPOTHESIS ABOUT INITIAL EVENTS IN PSORIASIS

The origin of the T cell deficiency could depend on many factors, particularly genetic and/or viral

Before introducing this last step it must be stressed that much of it is speculation beyond firm evidence, with the exception of genetic factors.

The genetic factors are obvious. There is a family pattern in 30% of cases (Farber & Nall, 1974). Homozygous twins are nearly always simultaneously involved (Watson *et al.*, 1972). In all likelihood, psoriasis has a multifactorial inheritance linked to several genes which could be related to the major histocompatibility complex (MHC).

The exact role of HLA antigens is not clear. However, various hypotheses have been raised:

The HLA phenotype could support the penetrance of different genes involved in the disease sensitivity.

The association between psoriasis and an HLA-B gene could be evidence of a linkage disequilibrium between this gene and the Ia region. Effectively, HLA antigens with a high frequency in psoriasis depend on the B locus, near the chromosome region bearing Ia genes (possible equivalent of I region on the H-2 mice chromosome). In animals this region is involved in antigen recognition, and in the T suppressor-T helper-B cell cooperation.

Otherwise, such genetic factors could also play a role by influencing the sensitiveness to a viral agent. Three hypotheses can be put forward:

A viral injury of thymus-dependent cell clones could result in the destruction of T-suppressor cells, either by a direct cytopathogenic effect, or by an immune reaction against the infected lymphocytes. Such a phenomenon could induce an exacerbation of the chronic viral infection, an increase of auto-antibody production by naturally auto-reactive B cells and the formation of skin-fixed immune complexes (Phillips, 1976).

Owing to a genetic predisposition, viruses could modify the membrane MHC antigens (Denman *et al.*, 1976; Oldstone *et al.*, 1976). Then, the normal T cells could interact with the 'incompatible' new-formed cells; this could generate many thymus-dependent activities: cytotoxic T cells, helper effect, lymphokine production.

Finally, a common viral infection, or a 'slow virus' infection, could induce, in subjects with a genetic susceptibility to produce immune complexes, the formation of persistent antigen-antibody complexes able to block the T-suppressor cells (Talal, 1976).

However, no direct proof of a virus in psoriasis is yet available. Prunieras *et al.* (1975) have described in psoriatic lymphocytes inclusions of Høvig's type (Hovig, Jeremic & Stavem, 1968); but their significance is far from sure.

A viral agent could act in other ways, for example the infection of nuclear material of epidermal cells, inducing an autoimmune reaction elicited by the antinuclear antibodies reported by Cormane *et al.* (1976).

It should be noted that genetic factors could be involved in other ways such as a congenital epidermal biochemical disturbance, as congenital cell membrane abnormalities, or as a congenital defect of dermal blood and lymphatic vessels. An abnormal vessel structure has been demonstrated in the psoriatic lesion, with numerous gaps in the vessel walls (Braverman & Yen, 1974). Such gaps have also been described in psoriatic basal lamina (Cox, 1969) and could permit auto-antibody migration into epidermis which could be responsible for the membrane lesions.

Besides this genetic predisposition many other extrinsic factors could play an important role in determining activity of the disease, such as factors directly interfering with membrane receptors (psoriasiform rashes from β blockers), psychological upsets (or stress) leading to important catecholamine secretion which could unmask the membrane receptor disequilibrium. Lastly, any factor modifying the immunological status could reveal a previously latent defect.

Of course, all these hypothesis are only speculative but they constitute numerous fields of work for the future.

CONCLUSION

The steps in the pathogenesis of psoriasis which could be used as a background for further work can be summarized into seven propositions:

- (1) Psoriasis is an epidermal proliferative disease.
- (2) Epidermal proliferation is the result of cyclic nucleotide and prostaglandin imbalance.
- (3) This imbalance may be due to keratinocyte membrane abnormalities.
- (4) Membrane abnormalities could result from immunological phenomena located in psoriatic epidermis.

(5) Abnormal immunological phenomena may be related to a cyclic dissociated impairment of thymus-dependent lymphocytes.

(6) The cyclic T-cell defect could depend on genetic and viral factors.

(7) Numerous extrinsic factors could interfere in the steps of the psoriasis pathogenic way, revealing a previously latent defect and thus resulting in the clinical disease.

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REFERENCES

- ASO, K., DENEAU, D.G., KRUGG, I., WILKINSON, D.I. & FARBER, E.M. (1975) Epidermal synthesis of prostaglandins and their effect on levels of cyclic adenosine 3'5' monophosphate. *Journal of Investigative Dermatology*, **64**, 326.
- BACH, M.A. & BACH, J.F. (1973) Studies on thymus products. VI. The effects of cyclic nucleotides and prostaglandins on rosette forming cells. Interaction with thymic products. *European Journal of Immunology*, **3**, 778.
- BARRIERE, H., LITOUX, P. & GERAUT, C. (1974) Etude des cellules cornées du psoriasis en microscopie électronique à balayage. *Dermatologica*, **149**, 257.
- BEUTNER, E.H., JABLONSKA, S., JARZABEK-CHORZELSKA, M., MARCIEJOWSKA, E., GENOWEDA, R. & CHORZELSKI, T. (1975) Studies in immunodermatology. VI. Immunofluorescence studies of autoantibodies to the stratum corneum and in vivo fixed IgG in stratum corneum of psoriatic lesions. *International Archives of Allergy and Applied Immunology*, **48**, 301.
- BRAVERMAN, I.M. & YEN, A. (1974) Microcirculation in psoriatic skin. *Journal of Investigative Dermatology*, **62**, 493.
- BRODY, I. (1962) The ultrastructure of the epidermis in psoriasis vulgaris as revealed by electron microscopy. *Journal of Ultrastructural Research*, **6**, 304.
- BULLOUGH, W.S. (1962) The control of mitotic activity in adult mammalian tissues. *Biologic Revue*, **37**, 307.
- BULLOUGH, W.S. & LAURENCE, E.B. (1964) Mitotic control by internal secretion. The role of the chalone-adrenalin complex. *Experimental Cell Research*, **33**, 176.
- CAPUTO, R., PELUCCHETTI, D. & INNOCENTI, M. (1977) Plasma membranes in psoriatic cells — A freeze-fracture study. *The European Society for Dermatological Research*, May 2 (oral communication).
- CLOT, J., CHARMASSEN, E., MASSIP, H., GUILHOU, J.J. & MEYNADIER, J. (1975) Deficit des T lymphocytes dans le psoriasis. *Nouvelle Presse Médicale*, **4**, 2039.
- CLOT, J., DARDENNE, M., BROCHIER, J., ANDARY, M. & GUILHOU, J.J. (1978). Evaluation of lymphocyte subpopulation and T cell functions in psoriasis. *Clinical Immunology and Immunopathology*, in press.
- CORMANE, R.H., HUNYADI, J. & HAMERLINK, F. (1976) The role of lymphoid cells and polymorphonuclear leucocytes in the pathogenesis of psoriasis. *The Journal of Dermatology*, **3**, 247.
- CORMANE, R.J., HAMERLINK, F., SIMON, M. & SIDDIQUI, A.H. (1977) Photo immunology in psoriasis. *The European Society for Dermatological Research*, Amsterdam, May. (oral communication).
- COX, A.J. (1969) The dermal epidermal junction in psoriasis. *Journal of Investigative Dermatology*, **53**, 428.
- DENMAN, A.M., PELTON, B.K., APPLEFORD, D. & KINSLEY, M. (1976) Virus infections of lymphoreticular cells and auto immune diseases. *Transplantation Review*, **31**, 99.
- ELGJO, K. (1975) Epidermal chalone and cyclic AMP: and in vitro study. *Journal of Investigative Dermatology*, **64**, 14.
- FARBER, E.M. & NALL, M.L. (1974) The natural history of psoriasis in 5600 patients. *Dermatologica*, **148**, 1.
- FLORIN-CHRISTENSEN, A., MALDONADO COCCO, J.A., ARANA, R., PORRINI, A., NOM, A. & GARCIA MORTEO, O. (1974) Anti-gamma-globulin factors in psoriasis. *Dermatologica*, **149**, 220.
- FRASER, N.G., DICK, H.M. & GRICHTON, W.B. (1969) Immunoglobulins in dermatitis herpetiformis and various other skin diseases. *British Journal of Dermatology*, **81**, 89.
- GEHA, R.S., COLTEN, H.R., SCHNEEBERGER, E. & MERLER, E. (1975) Cooperation between human thymus-derived

- and bone marrow-derived lymphocytes in the antibody response to ragweed antigen E *in vitro*. *Journal of Clinical Investigation*, **56**, 386.
- GELFANT, S. (1976) The cell cycle in psoriasis: a reappraisal. *British Journal of Dermatology*, **95**, 577.
- GLINSKI, W., HAFTEK, M., OBALAK, S. & JABLONSKA, S. (1977) Clinical aspects of T and B lymphocytes in psoriasis. *Archives for Dermatological Research*, **258**, 89.
- GOODWIN, P., HAMILTON, S. & FRY, L. (1974) The cell cycle in psoriasis. *British Journal of Dermatology*, **90**, 517.
- GUILHOU, J.J., MEYNADIER, J. & CLOT, J. (1973a) Presence de facteurs antigamma globuliniques sériques dans le psoriasis. *Nouvelle Presse Médicale*, **2**, 2750.
- GUILHOU, J.J., MEYNADIER, J., ROBINET-LEVY, M. & BASCOUL, S. (1973b) Modification des immunoglobulines sériques et des IgA salivaires dans le psoriasis. *Nouvelle Presse Médicale*, **2**, 3047.
- GUILHOU, J.J., CLOT, J., MEYNADIER, J. & LAPINSKI, H. (1976a) Immunological aspects of psoriasis. I. Immunoglobulins and anti-IgG factors. *British Journal of Dermatology*, **94**, 501.
- GUILHOU, J.J., CLOT, J., MEYNADIER, J., CHARMASSON, E., BROCHIER, J. & DARDENNE, M. (1976b) Immunological aspects of psoriasis. II. Dissociated impairment of thymus-dependent lymphocytes. *British Journal of Dermatology*, **95**, 295.
- GUILHOU, J.J. & MEYNADIER, J. (1976) Immunologie du psoriasis. *Annales de Dermatologie et de Syphiligraphie*, **103**, 509.
- HALPRIN, K.M. (1976) Cyclic nucleotides and epidermal cell proliferation. *Journal of Investigative Dermatology*, **66**, 339.
- HAMMARSTROM, S., HAMBERG, M., SAMUELSSON, B., DUELL, E., STAWISKI, M. & VOORHEES, J.J. (1975) Increased levels of free arachidonate PGE₂ and F₂ and 12 L Hydroxy 5-8-10-14 eicosatetraenoic acid (Hete) in uninvolved v-s involved psoriatic epidermis. Evidence for perturbed regulation of free arachidonate levels in lesions. *Clinical Research*, **23**, 3, 386 A.
- HARKONEN, M., HOPUSU-HAVU, U.K. & RAIJ, K. (1974) Cyclic-adenosine-monophosphate, adeny-cyclase and cyclic-nucleotide phosphodiesterase in psoriatic epidermis. *Acta dermato-venereologica*, **54**, 13.
- HEID, E., BIGEL, P., REUTER, G., FOUSSEAU, J. & BASSET, A. (1976) Données concernant l'immunité cellulaire dans le psoriasis. *Annales de Dermatologie et de Syphiligraphie*, **103**, 577.
- HOVIG, T., JEREMIC, M. & STAVEM, P. (1968) A new type of inclusion bodies in lymphocytes. *Scandinavian Journal of Hematology*, **5**, 81.
- HOWELL, F.A., CHAMBERLAIN, M.A., PERRY, R.A., TORFIGIANI, G. & ROITT, I.M. (1972) IgG antiglobulin levels in patients with psoriatic arthropathy, ankylosing spondylitis and gout. *Annals of the Rheumatic Diseases*, **31**, 129.
- JABLONSKA, S., CHORZELSKI, T., JARZABEK-CHORZELSKA, M. & BEUTNER, E.H. (1975) Studies in immuno-dermatology. VII. Four compartment system studies of IgG in biopsies of psoriasis and control dermatoses. *International Archives of Allergy and Applied Immunology*, **48**, 324.
- KROGH, H.K. (1970) The occurrence of antibodies to stratum corneum in man. *International Archives of Allergy*, **37**, 649.
- KROGH, H.K. & TONDER, O. (1972) Immunoglobulins and anti-immunoglobulin factors in psoriatic lesions. *Clinical and Experimental Immunology*, **10**, 623.
- KROGH, H.K. & TONDER, O. (1973) Antibodies in psoriatic scales. *Scandinavian Journal of Immunology*, **2**, 45.
- LEVANTINE, A. & BROSTOFF, J. (1975) Immunological response of patients with psoriasis and the effect of treatment by methotrexate. *British Journal of Dermatology*, **93**, 659.
- LISCHKA, G. (1976) T lymphocytes and psoriasis. *Archives for Dermatological Research*, **257**, 107.
- MAHRLE, G. & ORFANOS, C.E. (1974) Decreased membrane-bound ATP-hydrolytic activity in psoriatic epidermis. *British Journal of Dermatology*, **91**, 529.
- MAHRLE, G. & ORFANOS, C.E. (1975) Ultrastructural localisation and differentiation of membrane bound ATP utilising enzymes including adeny-cyclase in normal and psoriatic epidermis. *British Journal of Dermatology*, **93**, 495.
- MAHRLE, G. & ORFANOS, C.E. (1977) The plasma unit membrane. *British Journal of Dermatology*, **96**, 215.
- MARGHESCU, S. & BRAUN-FALCO, O. (1970) Quantitatives Verhalten von Immunoglobulinen und Komplement bei psoriasis. *Archiv für Klinische und Experimentelle Dermatologie*, **238**, 417.
- MEI MEI MUI, HSIA, S.L. & HALPRIN, K.M. (1975) Further studies on adeny-cyclase in psoriasis. *British Journal of Dermatology*, **92**, 255.
- OLDSTONE, M.B.A., HASPEL, M.V., PELLEGRINO, M.A., KINGSBURY, D.T. & OLDING, L. (1976) Histocompatibility complex and virus infection latency and activation. *Transplantation Review*, **31**, 325.
- OON, C.H., GOODWIN, P.G., KIND, P.R.N., SEAH, P.F. & FRY, L. (1973) Salivary IgA in patients with psoriasis and dermatitis herpetiformis. *Acta dermato-venereologica*, **53**, 340.
- ORFANOS, C.E. (1971) Scanning electron microscopy in psoriasis. In: *Psoriasis—Proceedings of the International Symposium, Stanford University* (Ed. by E.M. Farber and A.J. Cox), p. 169. Stanford University Press, California.

- ORFANOS, C.E., SCHAUMBUR-LEVER, G., MAHILE, G. & LEVER, W.F. (1973) Alterations of cell surfaces as a pathogenetic factor in psoriasis. Possible loss of contact inhibition of growth. *Archives of Dermatology*, **107**, 38.
- PENNEYS, N.S., ZIBOH, V., LORD, J. & SIMON, P. (1975) Inhibition of prostaglandin synthesis in psoriatic plaques. *Nature*, **254**, 351.
- PHILLIPS, P.E. (1976) Rheumatic disease during the course of known viral infections: models of the possible pathogenesis of rheumatoid arthritis. In: *Infection and Immunology in the Rheumatic Diseases*. (Ed. by D.C. Dumonde), p. 205. Blackwell Scientific Publications, Oxford.
- PRUNIERAS, M., GRUPPER, C., DUREPAIRE, R. & REGNIER, M. (1975) Examen du sang périphérique dans 100 cas dermatologiques. *Annales de Dermatologie et Syphiligraphie*, **102**, 33.
- RIMBAUD, P., MEYNADIER, J., GUILHOU, J.J. & CLOT, J. (1972b) A propos des troubles immunologiques et biochimiques dans le psoriasis. *Nouvelle Presse Médicale*, **42**, 2836.
- RIMBAUD, P., MEYNADIER, J., GUILHOU, J.J. & CLOT, J. (1973) Anti-IgG activity on peripheral blood lymphocytes in psoriasis. *Archives of Dermatology*, **108**, 373.
- RIMBAUD, P., MEYNADIER, J., GUILHOU, J.J., CLOT, J., SEIGNALET, J. & GUILHOU, E. (1974) Troubles immunitaires et antigènes d'histocompatibilité dans le psoriasis. *Annales de Dermatologie et de Syphiligraphie*, **101**, 359.
- ROTHBERG, S., CROUNSE, R.G. & LEE, J.L. (1961) Glycine C incorporation into the proteins of normal stratum corneum and abnormal stratum corneum of psoriasis. *Journal of Investigative Dermatology*, **37**, 497.
- RUSSEL, J.J., SCHULTES, L.M. & KUBAN, D.J. (1972) Histocompatibility (HL-A) antigens associated with psoriasis. *New England Journal of Medicine*, **287**, 740.
- STOBO, J.D. & PAUL, W.E. (1973) Functional heterogeneity of immune lymphoid cells III. Differential responsiveness of T cells to phytohaemagglutinin and concanavalin A as a probe for T cell subsets. *Journal of Immunology*, **110**, 362.
- SVEJGAARD, A. & RYDER, L.P. (1976) Interaction of HLA molecules with non immunological ligands as an explanation of HLA and disease associations. *Lancet*, **ii**, 547.
- TALAL, M. (1976) Disordered immunologic regulation and auto-immunity. *Transplantation review*, **31**, 240.
- VAN SCOTT, E.J. & EKEL, T.M. (1963) Kinetics of hyperplasia in psoriasis. *Archives of Dermatology*, **88**, 373.
- VARELZIDIS, A. & THEODORIDIS, A. (1971) Serum immunoglobulins in psoriasis before and after ultraviolet light therapy. *British Journal of Dermatology*, **85**, 14.
- VOORHEES, J.J. & DUELL, E.A. (1971) The possible role of the chalone mechanism in psoriasis. In: *Psoriasis, Proceedings of the International Symposium, Stanford University* (Ed. by E.M. Farber and A.J. Cox), p. 305. Stanford University Press, California.
- VOORHEES, J.J., DUELL, E.A., BASS, L.J., POWELL, J.A. & HARRELL, E.R. (1972) Decreased cyclic AMP in the epidermis of lesions of psoriasis. *Archives of Dermatology*, **105**, 695.
- VOORHEES, J.J., STAWISKI, M., DUELL, E.A., HADDOX, M.K. & GOLDBERG, N.D. (1973) Increased cyclic GMP and decreased cyclic AMP levels in the hyperplastic abnormally differentiated epidermis of psoriasis. *Life Science*, **13**, 639.
- VOORHEES, J.J., MARCELO, C.L. & DUELL, E.A. (1975) Cyclic AMP, cyclic GMP and glucocorticoides as potential metabolic regulators of epidermal proliferation and differentiation. *Journal of Investigative Dermatology*, **65**, 179.
- VOORHEES, J.J., CHAMBERS, D.A., DUELL, E.A., MARCELO, C.L. & KRUEGER, G.C. (1976) Molecular mechanisms in proliferative skin disease. *Journal of Investigative Dermatology*, **67**, 442.
- WATSON, W., CANN, M.M., FARBER, E.M. & NALL, M.L. (1972) The genetics of psoriasis. *Archives of Dermatology*, **105**, 197.
- WEINSTEIN, G.D. & FROST, P. (1968) Abnormal cell proliferation in psoriasis. *Journal of Investigative Dermatology*, **50**, 254.
- WEINSTEIN, G.D. & VAN SCOTT, E.J. (1965) Autoradiographic analysis of turnover times of normal and psoriatic epidermis. *Journal of Investigative Dermatology*, **45**, 257.
- WHITE, S.H., NEWCOMER, U.D., MICKY, M.R. & TERASAKI, P.I. (1972) Disturbance of the HL-A antigen frequency in psoriasis. *New England Journal of Medicine*, **287**, 740.
- WRIGHT, R.K., MANDY, S.H., HALPRIN, K.M. & HSIA, S.L. (1973) Defects and deficiency of adenyl cyclase in psoriatic skin. *Archives of Dermatology*, **107**, 47.
- WRIGHT, N.A. (1976) A new look at epidermal cell kinetics in psoriasis and other dermatoses. *Clinical and Experimental Dermatology*, **1**, 275.
- YOSHIKAWA, K., ADACHI, K., HALPRIN, K.M. & LEVINE, V. (1975a) On the lack of response to catecholamine stimulation by the adenyl cyclase system in psoriatic skin. *British Journal of Dermatology*, **92**, 619.
- YOSHIKAWA, K., ADACHI, K., HALPRIN, K.M. & LEVINE, V. (1975b) Is the cyclic AMP in psoriatic epidermis low? *British Journal of Dermatology*, **93**, 253.

The XVth International Congress of Dermatology

The XVth International Congress of Dermatology was held in Mexico City from 16 to 20 October, 1977 and was an outstanding success. It is no secret that the usefulness and indeed the viability of such meetings had in recent years been called very much into question. This congress will have done much to dispel any previous misgivings. It demonstrated that with an imaginative and well prepared programme, combined with friendly efficiency and hospitality, as in Mexico, such meetings are invaluable. In addition to the opportunity of hearing world authorities on many aspects of dermatology, appropriate discussion was actively encouraged. Informal meetings with speakers and with other colleagues were aided by the happy atmosphere and the admirable facilities of the Congress Centre.

The programme catered equally for practising dermatologists and for those with a main interest in basic sciences, and emphasized the relevance of research to clinical problems. The morning began with a demonstration of carefully selected transparencies, complete with a short history, of uncommon or geographically localized disorders. This was followed by a lecture given by a speaker of outstanding distinction in a branch of basic science relevant to dermatology. For the remainder of the morning members could choose between sessions on subjects of topical interest, symposia on recent advances, work-shops, and courses of instruction, the latter two continuing after a short break well into the afternoon. The general level of these sessions was very high and the attendance excellent. A few enthusiasts held informal discussion groups in the evening. There was an excellent exhibition of posters changed daily. Sessions on film projections and free communications were also available in the afternoons.

The main part of the meeting was held in the Congress Building of the Mexican Social Security Institute's National Medical Centre, which was designed specifically for large gatherings and which has attracted many international meetings in other branches of medicine. The social programme for members and for those accompanying them was extremely generous and gave a fascinating introduction to the history and arts of Mexico. For the great success and friendliness of this congress we are greatly indebted to our Mexican colleagues, Professor F. Latapi, the Honorary President of the Congress, Professor A. Gonzáles Ochoa, the Executive President, and Professor Luciane Dominguez, the General Secretary, and to all their many helpers, whose efficiency and kindness was matched only by their hospitality.

In the preparation of the professional programme by the International Committee of Dermatology and again in which our Mexican colleagues were so helpful it is invidious to single out individuals for special praise. There was general regret that Professor Felix Sager, who had been Secretary General Treasurer of the International Committee for the past five years with such diligence, was unable to be present at the meeting for reasons of health. Amongst all those who had taken part in the preparation for this congress there was universal agreement that the major credit for its success must go to Rudolf Baer, President of the International Committee for his unremitting efforts and his kindly skill at all times. It was indeed fitting that at the congress he should receive the Alfred Marchionini Gold Medal for his professional distinction and for his contribution to the encouragement of good fellowship amongst dermatologists throughout the world.

Professor Otto Braun-Falco and Professor Nils Thyresson were unanimously elected President and Secretary General Treasurer of the International Committee to prepare for the XVIth International Congress, which by general approval of all the delegates is due to be held in Tokyo in 1982 with Professor Yasuda as Honorary President and Professor Kukita as Executive President.

H.J. WALLACE

Book Reviews

Lehrbuch der Haut Krankheiten und Venerischen Infectionen für Studierende und Ärzte. By THEODOR NASEMANN and WOLFARD SAUERBREY (1974) Berlin: Springer Verlag. 300 Abb. XX, pp. 400. Price US \$19.70.

In this volume of some four hundred pages, there are approximately fifty pages devoted to venereology. The text is in double column, easy to read and well divided with headings and sub-headings. There is a profusion of tables, schematic drawings, photo-micrographs and clinical pictures. The photographs tend to be larger than life, and many examples of tumours and pictures of lupus vulgaris are so advanced as to be caricatures of the full blown disease, rather than the more mild and early forms, which are becoming relatively much more common.

There is no sparing of detail, and one is delighted to see that continental students in Germany are not presented with watered down versions 'for students and general practitioners', but instead here is a working manual of Modern Dermatology beautifully presented, which would satisfy many consultant dermatologists.

Lyell's disease, Sneddon Wilkinson disease, and references to pustular psoriasis of the Barber type are all here. The British dermatologist who acquires this volume will be well pleased with the contents, and the views expressed are familiar and acceptable. Viral disorders are especially well dealt with and Professor Nasemann is of course an expert in this field.

For Teachers of Dermatology, the classification of aetiology in skin diseases and the schematic drawings could well be adopted as refreshing new ways of teaching for our own students. The authors are to be congratulated.

MAURICE GARRETTTS

Cytostatica in der Dermatologie. By A. LUGER (1977) Berlin: Springer Verlag. 29 Abb. 10 Tab. XII, pp. 194. Price US \$10.10.

'Since the war', says the Author in his preface, 'external applications have not been so very important for Dermatologists. Antibiotics, antihistamines, corticosteroids, and now cytotoxic drugs have revolutionized the practice of Dermatology'. While not all British Dermatologists would allow external applications to be supplanted without protest, the cytotoxic group of drugs constitute a new type of weapon in the armamentarium against skin disease.

This slim volume approximately the size of an old fashioned Penguin book lists the common, as well as the more rarely used cytotoxic drugs, and their indication for use in dermatology and venereology, and possible side effects and dangers.

At the end of the work is a diagnostic index and the relevant cytotoxic drugs are listed for use in each disease where appropriate.

Most useful is the introductory chapter on the mode of action of the cytotoxic drugs and the point of action in the process of cell division of the various members of this group of substances. Some clear rationale can thus be achieved for the choice of drug and the dosage schedules recommended.

So much of the information is tabulated that the lack of a knowledge of German is no bar to opening this most interesting volume.

MAURICE GARRETTTS

Lecture Notes on Dermatology, 4th edn. By BETHEL SOLOMONS (1977) Oxford: Blackwell Scientific Publications. Pp. 271. Col. plates. B/W illus. Price £4.50.

This little book is widely used by medical students and will continue to be used although the price has increased to £4.50. The author states that he has revised this edition 'especially in regard to recent

innovations in treatment and the re-orientating of old concepts in relation to aetiology. The importance of immunological mechanisms is introduced as far as is pertinent. Many outmoded remedies have been discarded'.

On the whole these changes do appear in the text but it is surprising to find that under sedation for night, the barbiturates Tuinal and Nembutal are listed ahead of Mogadon.

I doubt if everyone would agree that a steroid cream should be applied singly or in conjunction with polythene coverings in psoriasis and it would be helpful if there was some discussion and awareness of the problems that may arise with such therapy. The unstable pustular variety of psoriasis should be mentioned.

The topical treatment of rosacea is often difficult but the use of steroid lotions should be restricted to the weakest preparations if used at all.

It is so difficult to convey a large condensation of fact to the reader and still create excitement and interest when anatomy and physiology of the skin are presented and this book like so many others could do more to present functional information which would help the reader understand more easily the subsequent chapters on disease.

'Lecture Notes' is a useful pocket sized dermatology text book which lists and describes the most common conditions including leprosy and syphilis and has a large collection of both black and white and colour photographs which are of good quality.

R.A. MAIN

Correspondence

Different pathogenesis for psoriatic reaction to lithium and practolol?

SIR, Reports in recent years on adverse cutaneous reactions more or less similar to psoriasis and provoked by practolol have received widespread attention. Recently lithium for psychiatric disease has also been associated with exacerbation of psoriasis (Baker, 1977; Bakker & Pepplinkhuizen, 1976; Carter, 1972; Skott, *et al.*, 1977). To our knowledge, 22 such cases have already been reported in detail. On the basis of the mounting number of patients with lithium-triggered psoriatic reactions, we believe it is appropriate to compare their clinical features with those in the practolol cases.

(1) Clinically, the practolol reactions were mostly psoriasiform but lichenoid and lupus-erythematosus-like rashes and exfoliative dermatitis have also been seen. In most reported cases no previous history of psoriasis is known, although exacerbation of a pre-existing psoriasis has been reported (Söndergaard *et al.*, 1976). As for lithium, our 7, Carter's 3 and 3 of Bakker *et al.*'s 4 patients had aggravation of psoriasis. A few patients with psoriasiform lesions erupting during lithium therapy have also been reported from Holland (Bakker & Pepplinkhuizen, 1976), Denmark (Thormann, 1977) and Sweden (Swedish adverse drug reaction committee, 1977).

(2) In the psoriasiform type of practolol rash, the occurrence of hyperkeratosis on the soles and palms is a characteristic and early sign (Felix, Ive & Dahl, 1974). In the lithium cases, the clinical picture was that of an extensive psoriasis with less response to traditional therapeutic modalities; none of our 7 patients had palmoplantar lesions. Contrary to the practolol cases, ocular lesions have not been reported in the lithium patients.

(3) The average time before skin lesions appeared was 29 months in 15 patients with cutaneous lesions from practolol (Behan *et al.*, 1976). Felix *et al.* (1974) reported that 10 of 14 patients with the psoriasiform type of practolol rash had a latent period longer than 8 months (mean 13 months). In 3 patients with previous psoriasis, the mean time before the exacerbation of psoriasis was 17 months, and in 8 patients without previous psoriasis the corresponding figure was 15 months (Söndergaard *et al.*, 1976). As for lithium, the skin lesions worsened within the first weeks to 2–4 months in cases reported by Carter, Bakker & Pepplinkhuizen and Skott *et al.* In a recent Danish series of 8 patients, the latent period was 2–12 months with a mean of 5 months (Thormann, 1977).

(4) Immunological abnormalities are common in patients with practolol reactions. Dahl *et al.* (1975) found circulating anti-nuclear antibodies in 42% of the patients and Behan *et al.* in 53%. Actually 87% were positive for at least one of the auto-antibodies (Behan *et al.*, 1976). The significance of these findings for the development of skin lesions is unknown at present. An incidence of antinuclear factor of 18% in a series of lithium treated psychiatric patients has been reported (Presley, Kahn & Williamson, 1976).

We have now examined 5 of our patients for rheumatoid factor, auto-antibodies to cell nuclei, thyroid antigens, gastric parietal cell, smooth muscle, epidermal intercellular material and basement membrane zone (in serum), and serum DNA binding capacity. The results were normal in all tests.

The number of patients with psoriasis probably exacerbated by lithium is still small, but the available data indicate that there may be different mechanisms of action responsible for the cutaneous reactions to lithium and to practolol. Besides, it is interesting to notice that all 4 of our patients who were HLA-typed had one HLA-phenotype known to be associated with psoriasis (HLA-B13, -B13, -Bw17, -Bw-17). The interrelations of lithium and psoriasis should be studied in a large group of subjects on a prospective basis.

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REFERENCES

- BAKER, H. (1977) Koebner: Lithium. *International Psoriasis Bulletin*, **4**, no 3.
- BAKKER, J.B. & PEPPLINKHUIZEN, L. (1976) More about the relationship of lithium to psoriasis. *Psychosomatis*, **17**, 143.
- BEHAN, P.O., BEHAND, W.M.H., ZACHARIAS, F.J. & NICHOLLS, J.T. (1976) Immunological abnormalities in patients who had the oculomucocutaneous syndrome associated with practolol therapy. *Lancet*, **ii**, 984.
- CARTER, T.N. (1972) The relationship of lithium carbonate to psoriasis. *Psychosomatis*, **13**, 325.
- DAHL, M.G.C., FELIX, R.H., IVE, F.A. & WILKINS, D.S. (1975) Immunological aspects of practolol induced lupus erythematosus. *European Society for Dermatological Research*, Amsterdam, June 1975.
- FELIX, R.H., IVE, F.A. & DAHL, M.G.C. (1974) Cutaneous and ocular reactions to practolol. *British Medical Journal*, **iv**, 321.
- PRESLEY, R.B., KAHN, A. & WILLIAMSON, N. (1976) Antinuclear antibodies in patients on lithium carbonate. *British Medical Journal*, **ii**, 280.
- SKOTT, A., MOBACKEN, H. & STARMARK, J.E. (1977) Exacerbation of psoriasis during lithium treatment. *British Journal of Dermatology*, **96**, 445.
- SÖNDERGAARD, J., WADSKOV, S., ERENLUND-JENSEN, H. & MIKKELSEN, H.I. (1976) Aggravation of psoriasis and occurrence of psoriasiform cutaneous eruptions induced by practolol (Eraldin). *Acta dermato-venereologica*, **56**, 239.
- THORMANN, J. (1977) Psoriasis and lithium. **21st Nordic Dermatologic Congress**, Aarhus (Denmark), June 1977.

News and Notices

University Hospital of Wales, Cardiff

A Course on dermato-pathology will be held at The University Hospital of Wales, Cardiff, on Tuesday 19 September to Friday 22 September 1978, run jointly by the departments of dermatology and pathology. It is intended for dermatologists and pathologists in training.

Further information and application forms may be obtained from: Dr A.G.Knight, Consultant Dermatologist, Department of Dermatology, University Hospital of Wales, Heath Park, Cardiff CF4 4XW.

European Society for Comparative Skin Biology

European Society for Comparative Skin Biology conference will be held on 19–22 September 1978, at University College Hospital Medical School. The meeting will include short contributed papers, a poster exhibition, discussion group meetings on keratins and keratinization, and on cell growth and proliferation, a special lecture by Professor N.A.Mitchison, FRS, on the skin in immunology. Papers with abstracts will be accepted in the fields of cell structure and function, permeability and barrier function, and control mechanisms. Details from the local secretary: Dr P.A.Riley, Department of Biochemical Pathology, University College Hospital Medical School, University Street, London WC1E 6JJ.

Abstracts should be received preferably by May 1978.

Linnean Society of London in association with the British Association of Dermatologists

An international symposium on the skin of vertebrates will be held on 25–27 September 1978, at Queen Elizabeth College (University of London), Campden Hill Road, London W8.

Details from Mr T.O'Grady, Linnean Society of London, Burlington House, Piccadilly, London W1V 0LQ.

Convenors: Dr R.I.C.Spearman and Dr P.A.Riley.

Congres de l'Association des Dermatologistes et Syphiligraphes de Langue Française

The next meeting of this association will be held in Tunis on 1–3 June 1979. Main themes include 'Peau et Lumieres' and 'Ichtyoses'.

Further details can be obtained from: Professeur M.Ridha Gharbi, Service de Dermatologie, Hôpital Charles-Nicolas, Tunis, Tunisie.

5th Iranian Congress of Dermatology

The 5th Iranian Congress of Dermatology will be held in Razi Hospital, Tehran, on 30 May–1 June 1978. The main topics are 'Dermatology in the Middle East' and 'Therapeutic problems in dermatology'.

Further details may be obtained from: Dr B.V.Seyedi, Director of 5th Iranian Congress of Dermatology, The Razi Hospital, Shahpour Ave., Tehran, Iran.

Second Ain Shams Medical Congress

Ain Shams Faculty of Medicine announces its 'Second Ain-Shams Medical Congress' to be held in Cairo from 21-25 October, 1978. This congress will include 3 days for all branches of medicine and 2 days for Immunology.

Application forms and detailed information will be sent on request to: Professor Aly Abdel-Fattah, Secretary General, Ain Shams Faculty of Medicine, Abbassia—Cairo. P.O. Box 38, Abbassia.

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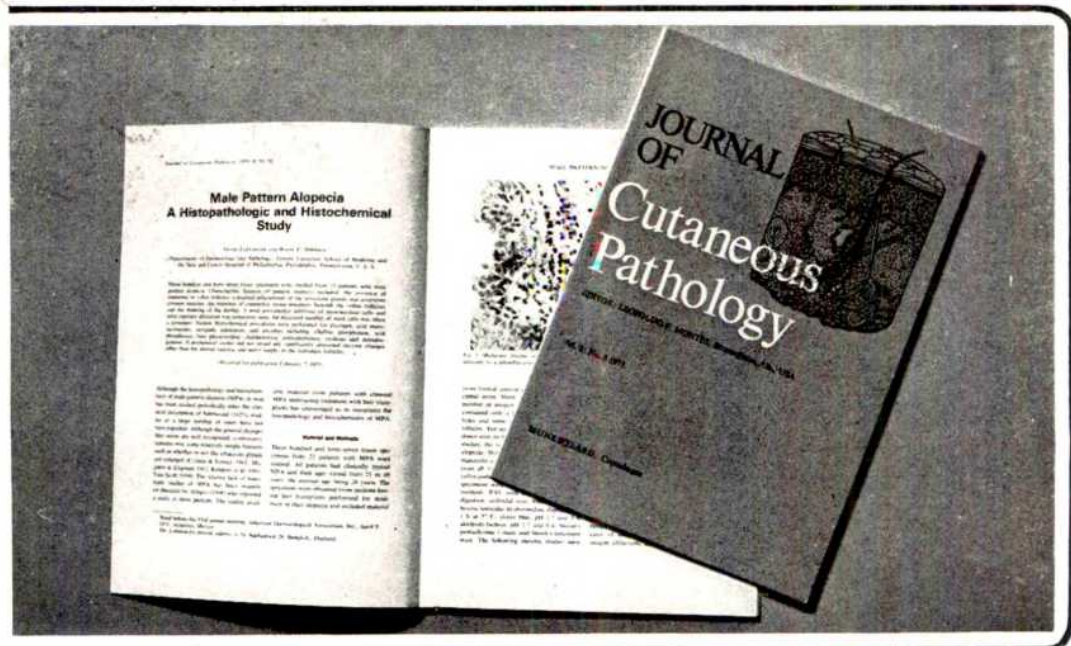
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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
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microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on faint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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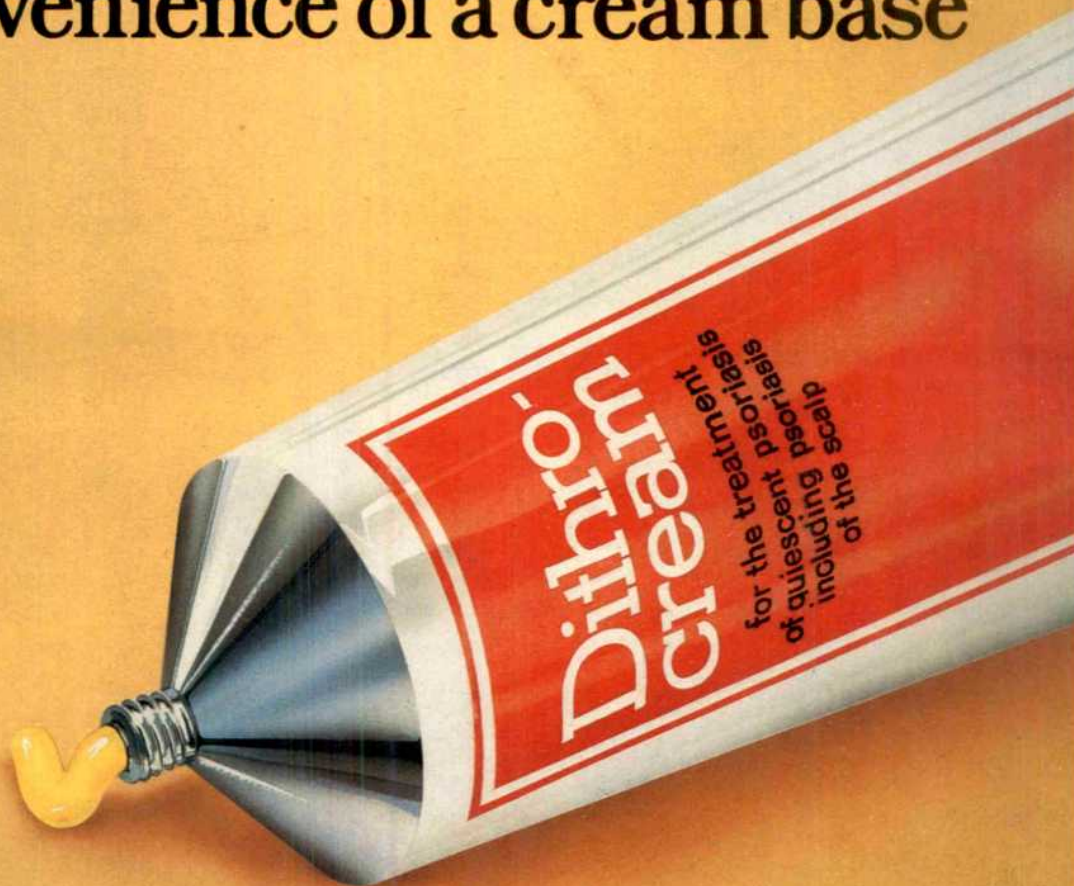
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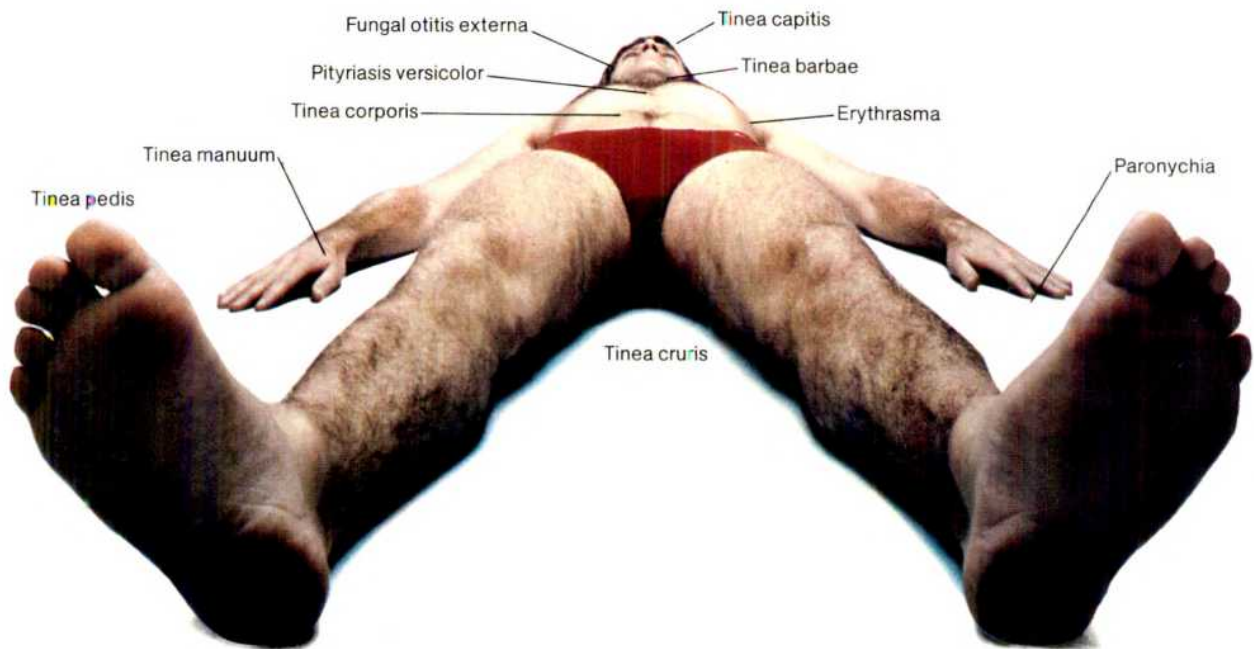
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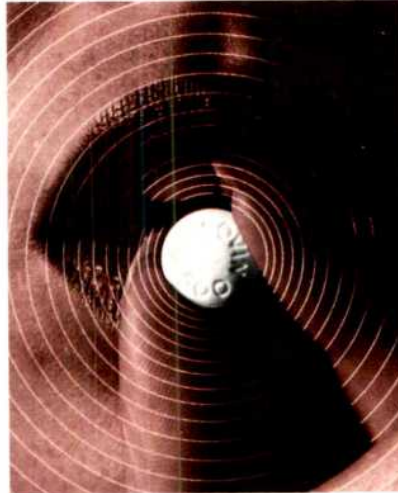
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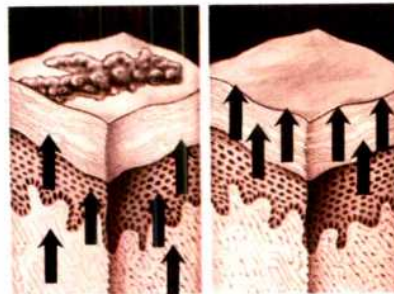


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Contra-indications

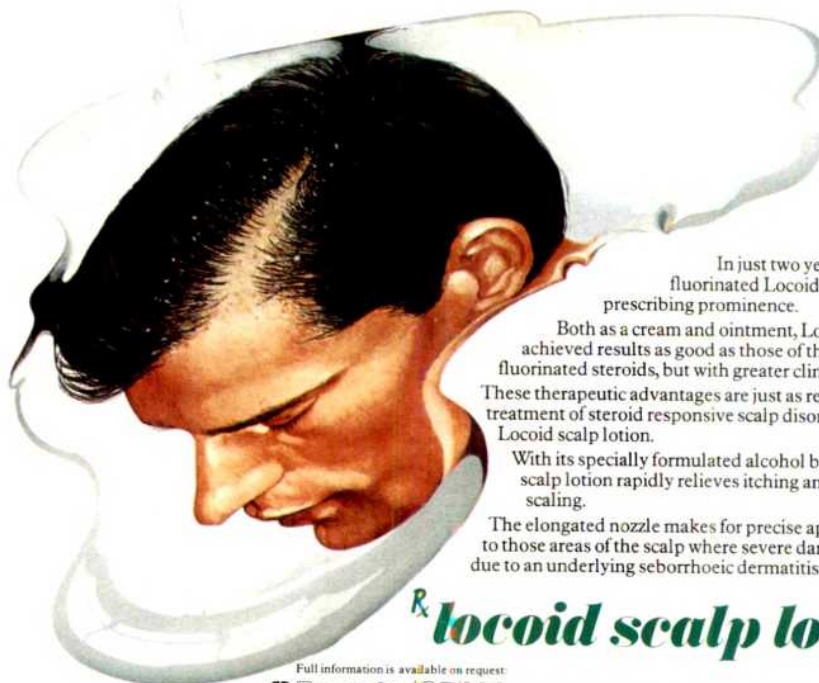
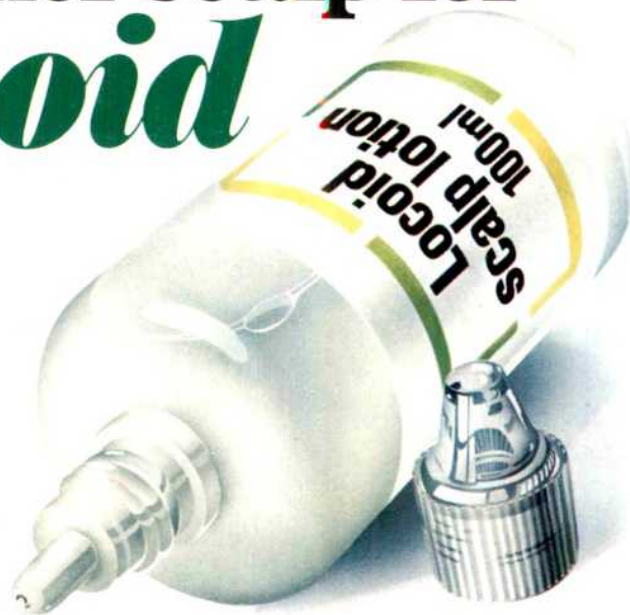
Porphyria or severe liver disease. Griseofulvin may cause

deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions

Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established.

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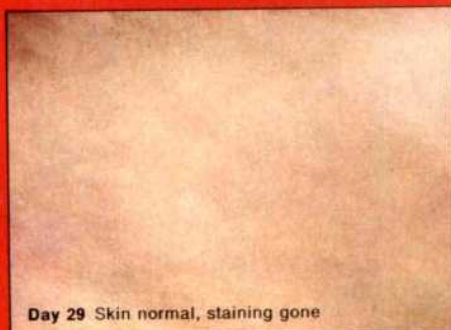
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General With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.

Rarely, treatment of psoriasis with corticosteroids for its withdrawal is thought to have provoked the pustular form of the disease.

Precautions

Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy. With all corticosteroids, prolonged application to the face is undesirable.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

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Product licence numbers
Ointment — PL/0004/0265 Cream — PL/0004/0266

Prescribing information

Dosage and administration

Apply to the affected area up to four times a day.

Contra-indications

Viral (e.g., herpes simplex) and dermatophyte (tinea/ringworm) diseases of the skin, and tuberculous lesions.

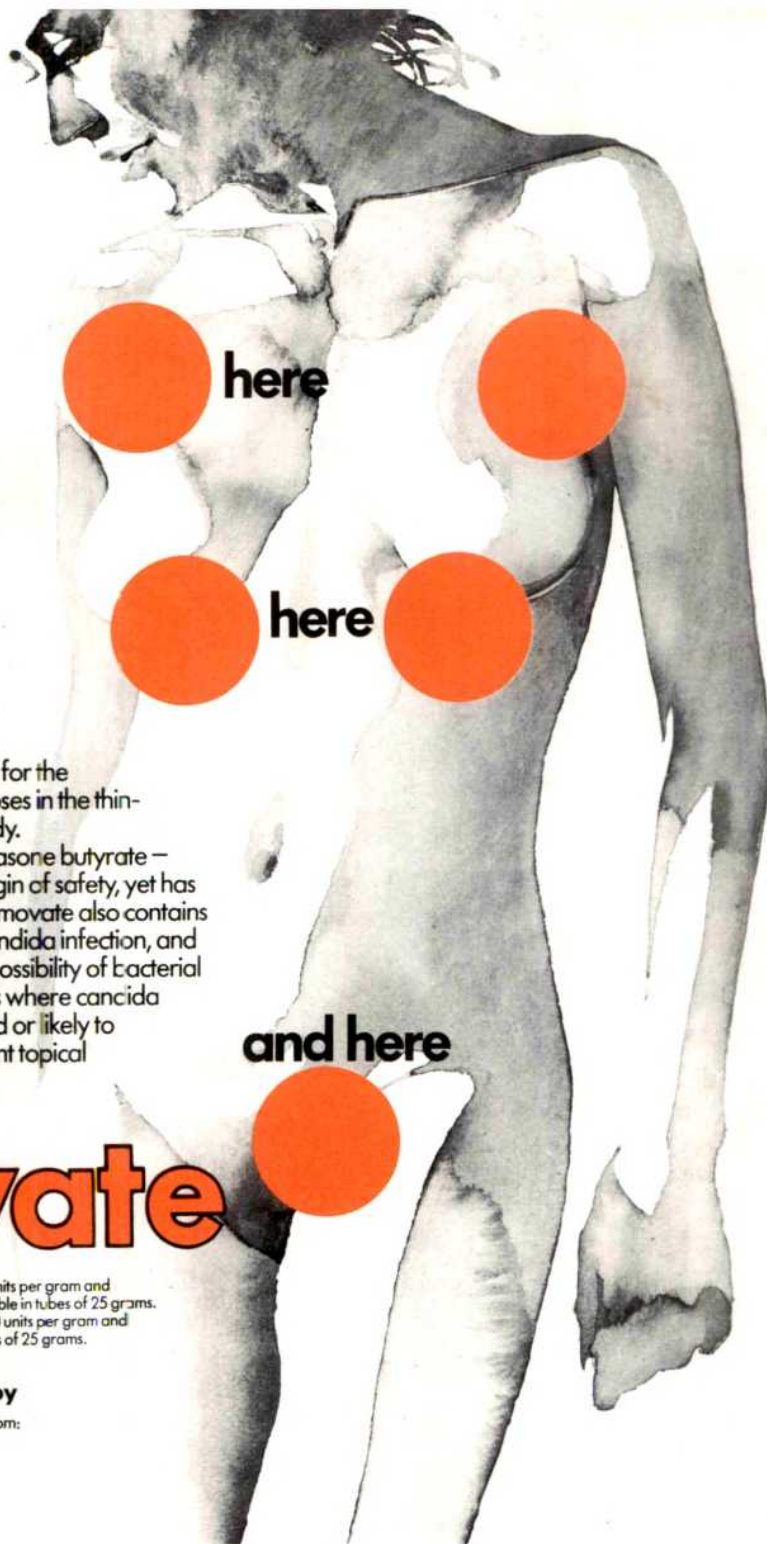
Precautions

In infants and children, long-term continuous topical steroid therapy should be avoided where possible, as adrenal suppression can occur even without occlusion. In infants, the napkin may act as an occlusive dressing and increase absorption. Any spread of

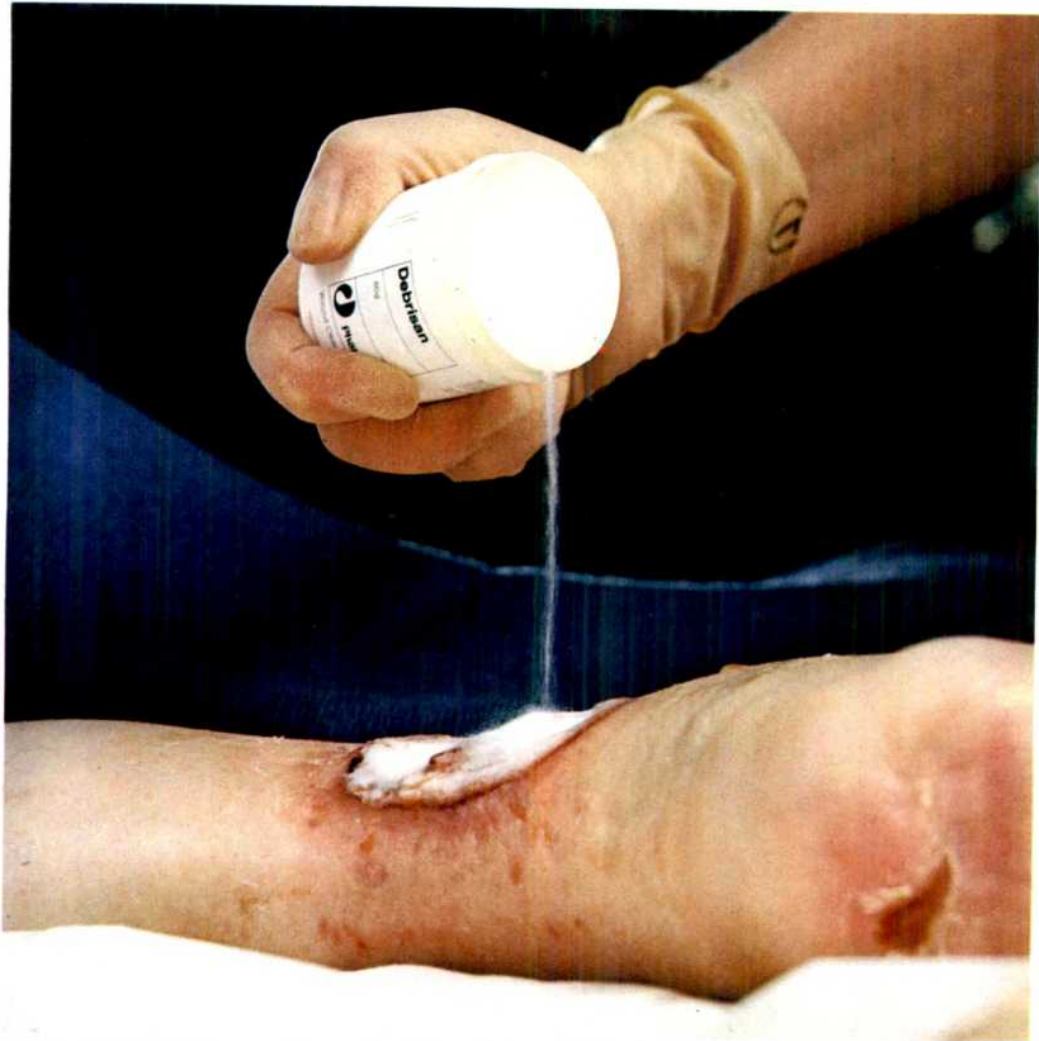
infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic chemotherapy. With all corticosteroids, prolonged application to the face is undesirable. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately. If large areas of the body were to be treated with Trimovate, it is possible that some patients would absorb sufficient steroid to cause transient adrenal depression despite the low degree of systemic activity associated with clobetasone butyrate. Local atrophic changes could possibly occur in situations where moisture increases absorption of clobetasone butyrate, but only after prolonged use.



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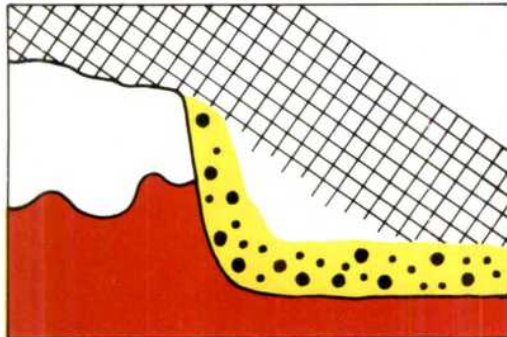
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Conventional dressing

Passive * partial *
sometimes painful



Top and bottom left

A conventional dressing may bridge the wound and hold a layer of pus against the wound surface.

Red: Oedema

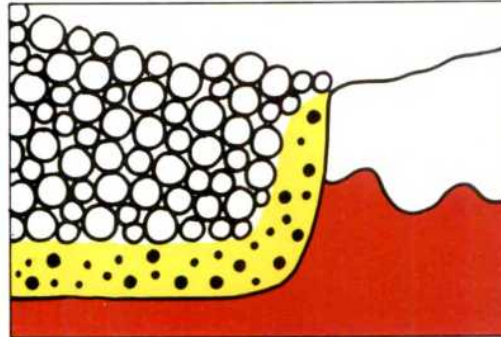
Yellow: Infected pus

Large black dots: Microorganisms and large molecules

Small black dots: Small molecules

Debrisan

active * continuous *
rapid relief of pain



Top right

Debrisan fills the wound achieving intimate contact with the wound surface.

Bottom right

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Indications

All kinds of secreting, suppurative wounds, such as post-operative wounds, leg ulcers, pressure sores, traumatic wounds, hand burns, suppurative ulcers, infected radio dermatitis, non-venereal penile ulcers.

Warning:

Debrisan spillage can render surfaces very slippery. Clear spillages promptly.

Caution:

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Debrisan-healing through cleansing



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lipid phase analogous to human
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PRODUCT
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Lichen planus: study with anti-human T lymphocyte antigen (anti-HTLA) serum on frozen tissue sections

ANNA ALARIO, JEAN-PAUL ORTONNE,
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Accepted for publication 2 December 1977

SUMMARY

The lymphocytic infiltrate of lichen planus was studied by the identification of the human T lymphocyte antigen in frozen tissue sections using specific anti-human T lymphocytes antigen antiserum. The infiltrate was shown to be composed principally of T lymphocytes.

Light microscopic examination of lichen planus (LP) reveals an intense band-like infiltrate of lymphocytes immediately below the epidermis. Immunological study of living cells mechanically liberated from the dermal infiltrate indicates that most of the infiltrating cells are T lymphocytes (Tan, Byrom & Hayes, 1975). Furthermore, on frozen sections, most of the infiltrate cells do not fix sheep erythrocytes sensitized with IgM antibody and complement (EAC) or with IgG antibody (EA) (Walker, 1976). This report concerns the *in situ* immunocytochemical identification by anti-human T lymphocyte antigen (HTLA) serum of the infiltrate on frozen tissue sections.

MATERIALS AND METHODS

Patients. Clinical data are summarized in Table 1. From each, a skin biopsy of approximately 0.6 cm was taken under local anaesthesia and divided into separate portions for routine histological and immunocytochemical study.

TABLE 1. Patients studied

Patient	Age	Sex	Histopathological diagnosis	Duration	Biopsy site
I	49	F	LP	2 years	Arm
II	35	F	LP	1 month	Back

Reprints requests: Dr A. Alario, FRA INSERM no. 11, 'Recherche Dermatologique et Immunologie', Clinique Dermatologique, Hôpital Edouard Herriot, 69374 Lyon, Cédex 2, France.

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Histopathology. The specimen was fixed in 3% formal calcium (Baker) and stained with H & E.

Immunoperoxidase

(a) *Study of surface Ig on frozen sections.* Sections 4 μ m thick were taken from specimens frozen in liquid nitrogen. After washing a direct reaction was carried out using a Fab anti-human Ig antiserum, prepared in sheep and conjugated with peroxidase (Institut Pasteur).

(b) *Study of in situ HTLA on sections.* The reaction was carried out using an indirect method. Four μ m cryostat sections were fixed by 2% paraformaldehyde, washed, and the HTLA was specifically marked with rabbit antiserum (Touraine *et al.*, 1974; Touraine *et al.*, 1976). The specificity of the anti-HTLA antiserum had been established and standardized commercially (Institut Merieux, Lyon). The rabbit Ig was then demonstrated by a Fab anti-rabbit Ig sheep peroxidase conjugate (Institut Pasteur). The peroxidase was revealed by Graham-Karnowsky medium (Graham-Karnowsky, 1966). The observations were carried out without counter-staining.

Controls. These tests were carried out on the same material using a control rabbit serum for the indirect reaction.

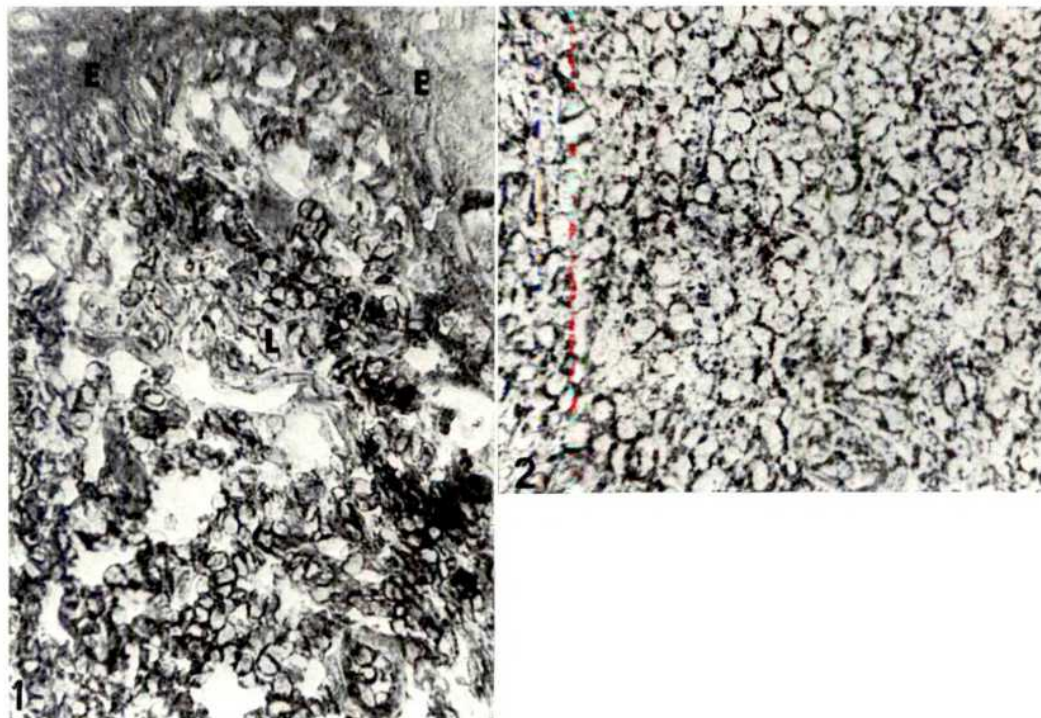


FIGURE 1. Labelling on the section by an anti-HTLA serum, revealed in the indirect reaction by a peroxidase conjugate. The labelling is seen only in the lymphocytic superficial skin infiltrate. E, epidermis; L, lymphocytes ($\times 300$).

FIGURE 2. All lymphocytes are labelled. The labelling appears as a granular pattern at the periphery of the cells ($\times 560$).

RESULTS

The topography of lymphoid cells marked with the anti-HTLA serum (Fig. 1) was identical to that of the typical superficial dermal infiltrate seen by routine histopathology in L.P. At high magnification (Fig. 2) most of the lymphoid cells are seen to be labelled. Labelling appeared as a granular pattern at the periphery of the cells.

DISCUSSION

T lymphocytes were identified using the E rosette technique (Wybran, Carr & Fudenberg, 1972). This method is inapplicable for tissue lymphocytes (Chused *et al.*, 1974; Jaffe *et al.*, 1974; Tannebaum *et al.*, 1975; Walker, 1976). Recently a T subpopulation specific differentiation antigen has been seen in the corresponding antiserum (Brochier *et al.*, 1976; Touraine *et al.*, 1974, 1976). Using this antiserum we were able to demonstrate T cell subpopulations on frozen tissue sections, obviating the need for cell extraction.

Our results showed that most of the cells of the infiltrate of L.P. were lymphocytes carrying HTLA antigen on the surface, and therefore of a thymus dependant nature. A quantitative analysis was carried out by a study on cells extracted mechanically from the infiltrate of biopsies. However, such technology (Tan *et al.*, 1975) is not exempt from criticism, as certain cells may be more easily extracted than others.

The identification of T lymphocytes is impossible on sections using the E rosette technique; Walker (1976) determined a preponderance of T lymphocytes in the lichen planus infiltrate by indirect evidence only. EA and EAC rosette methods on skin sections do not allow identification of the nature of the labelled cells. Our study allowed direct identification of the specific T lymphocyte antigens. Furthermore, we were able to establish precise morphological and immunological correlations.

Our results may be interpreted as an indicator of a cell-mediated immune reaction to unidentified cutaneous antigens in LP.

ACKNOWLEDGMENT

The excellent technical assistance of Miss D. Germain is acknowledged. This work was supported by C.N.R.S. (A.I. no. 031385).

REFERENCES

- BROCHIER, J., ABOU HAMED, Y.A., GUEHO, J.P. & REVILLARE, J.P. (1976) Study of human T and B lymphocytes with heterologous antisera. I. Preparation, specificity and properties of antisera. *Immunology*, **31**, 749.
- CHUSED, Y.M., HARDIN, J.A., FRANK, M.M. & GREEN, I. (1974) Identification of cells infiltrating the minor salivary glands in patients with Sjögren's syndrome. *Journal of Immunology*, **112**, 641.
- GRAHAM, R.C. & KARNOVSKY, M.J. (1966) The early stages of absorption of injected horseradish peroxidase in the proximal tubule of mouse kidney: Ultrastructural cytochemistry by a new technique. *Journal of Histochemistry and Cytochemistry*, **14**, 291.
- JAFFE, E.S., SHEVACH, E.M., FRANK, M.M., BERARD, C.W. & GREEN, I. (1974) Nodular lymphoma—evidence for origin from follicular B lymphocytes. *New England Journal of Medicine*, **290**, 813.
- TAN, R.S., BYROM, N.A. & MAYES, J.P. (1975) A method of liberating living cells from the dermal infiltrate. Studies on skin reticuloses and lichen planus. *British Journal of Dermatology*, **93**, 271.
- TANNENBAUM, H., PINKUS, G.S., ANDERSON, L.G. & SCHUR, P.H. (1975) Immunologic characterization of the mononuclear cell infiltrate in rheumatoid synovia, rheumatoid nodules and Sjögren's lip biopsies. *Arthritis and Rheumatism*, **18**, 305.

- TOURAINÉ, J.L., TOURAINÉ, F., KISZKISS, D.F., CHOI, Y.S. & GOOD, R.A. (1974) Heterologous specific antiserum for identification of human T lymphocytes. *Clinical and Experimental Immunology*, **16**, 503.
- TOURAINÉ, J.L., TOURAINÉ, F., DE BOUTEILLER, O. & BONNEAU, M. (1976) Identification et énumération des lymphocytes T et B chez l'homme en pratique quotidienne. In: *Techniques de Séparation et d'Identification des Lymphocytes Humains*. INSERM, 57, 121.
- WALKER, D.M. (1976) Identification of subpopulations of lymphocytes and macrophages in the infiltrate of lichen planus lesions of skin and oral mucosa. *British Journal of Dermatology*, **94**, 529.
- WYBRAN, J., CARR, M.C. & FUDENBERG, H.H. (1972) The human rosette forming cell as a marker of a population of thymus-derived cells. *Journal of Clinical Investigation*, **51**, 2537.

Identification of mononuclear cells *in situ* in skin lesions of lichen planus

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Accepted for publication 9 December 1977

SUMMARY

Cryostat sections of lesional skin of lichen planus were examined for receptors for sheep erythrocytes (T lymphocytes), Fc γ and C3b receptors (B lymphocytes and macrophages) and C3d (B lymphocytes). The indicator cells were applied using a closed chamber technique. The combination of the closed chamber technique and treatment of the sheep erythrocytes with a strong sulphhydryl reagent enabled us to detect T lymphocytes by their membrane receptors *in situ*.

T lymphocytes were the predominant mononuclear cells in the dermal infiltrate, with less macrophages and only a few B lymphocytes.

In lichen planus the most striking histological finding is a dense, band-like mononuclear cell infiltrate in the upper dermis. Recent reports have claimed that this mononuclear cell infiltrate consists predominantly of T lymphocytes (Tan, Byrom & Hayes, 1975; Walker, 1976). However, this has not yet been positively shown *in situ* because of inadequate techniques for the demonstration of T lymphocytes in tissue sections.

We have used a modified technique with a closed chamber to identify T lymphocytes directly in skin sections. In addition, human B lymphocytes and macrophages were identified by their different membrane receptors. B lymphocytes possess receptors for the complement fragments C3b and C3d, the latter being a fragment of C3b (Ross *et al.*, 1973). Macrophages have receptors for C3b, but not for C3d (Reynolds *et al.*, 1975). Both cell types also possess a receptor for the Fc of IgG (Abramson *et al.*, 1970; Dickler & Kunkel, 1972) (Table 1). The results of our studies are presented.

MATERIALS AND METHODS

Ten patients with skin lesions of lichen planus were studied. The patients had not received either local or general treatment. The diagnosis was confirmed by routine histology at the Department of Pathology.

Correspondence: Dr J.R.Bjerke, Department of Dermatology, N-5016 Haukeland sykehus, Norway.

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TABLE 1. The presence of various membrane receptors on mononuclear cells in tissue section

Cell	Indicator systems*			
	E	EA	EAC ₃ b	EAC ₃ d
T lymphocyte	+	—	—	—
B lymphocyte	—	+	+	+
Macrophage	—	+	+	—

* E=sheep erythrocytes; EA=sheep erythrocytes sensitized with rabbit IgG antibody; EAC₃b=sheep erythrocytes sensitized with rabbit IgM antibody and C₃b; EAC₃d=EAC₃b treated with C₃b inactivator.

+, receptor present; —, receptor not present.

Sera

Fresh normal human sera from blood donors were used as the source of complement (C). C was inactivated by heating at 56°C for 30 min. Inactivated sera absorbed with sheep erythrocytes were used as C₃b inactivator reagent (Ross & Polley, 1975).

Antisera to sheep erythrocytes were raised by immunization of rabbits, and samples were collected at various intervals after immunization to obtain IgM and IgG antibodies.

Tissues

Skin biopsies from lesions and unaffected areas were taken under local anaesthesia. The specimens were snap frozen on dry ice, and tissue sections, 4–6 µm thick, cut on a cryostat. Sections of specimens from normal spleen and kidney were used as control for the various indicator cells.

Demonstration of E receptors

Sheep erythrocytes (E) were treated with 2-aminoethylisothiuronium bromide hydrobromide (AET) (Sigma, St Louis, Mo., U.S.A.) as described by Kaplan & Clark (1974). The indicator cells were used as a 1% suspension in phosphate-buffered saline, pH 7.2 (PBS) containing 25% fetal bovine serum, stored at 4°C and used within 5 days.

For demonstration of E receptors in tissue sections a closed chamber technique was used (Tønder, Morse & Humphrey, 1974).

Cryostat sections of human spleen were included as controls in each experiment. T lymphocytes occupy preferentially the periarteriolar sheaths in the white pulp (Tønder *et al.*, 1974).

Some experiments were also performed with untreated sheep erythrocytes.

Demonstration of Fcγ receptors

The indicator cells (EA) were prepared by sensitizing E with rabbit IgG antibodies (A) in various amounts, expressed as agglutinating units. One agglutinating unit is defined as the amount of the highest dilution of antiserum which agglutinates an equal amount of 1% suspension of E. The washed EA were used as a 1% suspension in PBS as described by Milde & Tønder (1968) using a closed chamber technique. In strongly positive reactions (3+), the reacting tissue was completely covered by erythrocytes. In moderately (2+) and weakly (1+) positive reactions, partial covering of the section was observed. If no erythrocytes attached to the tissue, the reaction was termed negative (—).

Demonstration of complement receptors

The method was the same as described by Matre & Tønder (1976). E were sensitized with $\frac{1}{2}$ agglutinating unit of rabbit antibodies (A) of IgM class (EA). Zymosan-treated human sera, which retain considerable C3 activity with low C5 activity (Wong & Wilson, 1975), were used as source of C. The indicator cells (EAC3b) were used as a 1% suspension.

Cell-bound complement C3b is cleaved by C3 inactivator in serum to a fluid phase fragment C3c, and the cell-bound component C3d remains attached (Ruddy & Austen, 1971). To split cell-bound C3b, 1 ml of a 1% suspension of EAC3b was mixed with 2 ml of C3b inactivator reagent, incubated at 37°C for 2–4 h and used as a 1% suspension in a closed chamber.

Before each experiment the reactivity of the indicator cells was tested with tissue sections from human spleen and kidney. The EAC3b indicator cells adhere both within the white and the red pulp, whereas EAC3d adhere only to the white pulp (Christensen *et al.*, 1978). Only EAC3b adhere to the glomeruli in human renal tissue (Matre & Tønder, 1976). An additional control of reactivity for the two test systems is the immune adherence test (Atkinson & Frank, 1974; Reynolds *et al.*, 1975). EAC3b induce immune adherence of normal primate erythrocytes, while EAC3d indicator cells do not. The EAC3d cells were not used in tests with tissue section unless they were non-reactive in the immune adherence test. Tests with EA (IgM) served as negative controls for non-specific binding of indicator cells.

Fixation and staining

The microculture slides were placed for fixation overnight at 4°C in a solution of 0.5% glutaraldehyde and 1% paraformaldehyde in PBS (Matre & Tønder, 1976). After fixation the coverslip was carefully removed, gently washed in PBS and dried.

For staining of the indicator cells the sections were first treated for 20 min with benzidine to enhance the colour contrast (R. Matre, personal communication). Five mg of benzidine were dissolved in 10 ml 0.05% TrisHCl, pH 7.6. Immediately before use 0.2 ml H₂O₂ was added. The sections were then washed in PBS, stained with haematoxylin and eosin and mounted on slides.

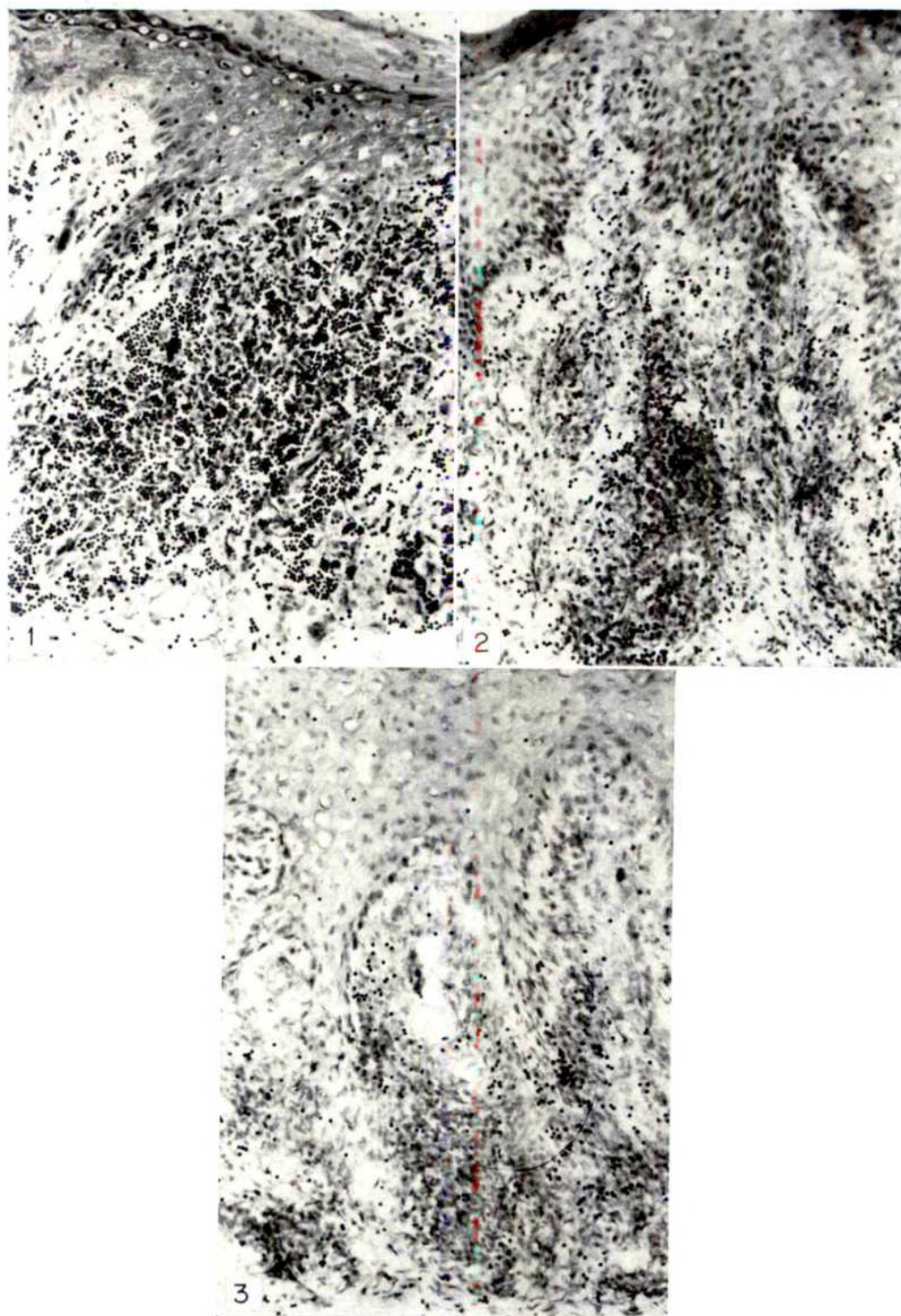
RESULTS

Sections of lesional skin absorbed AET-treated E onto the dermal mononuclear cell infiltrate in all cases (Fig. 1). The amount of E attached corresponded to the degree of the cell infiltrate in each case. The normal controls included were negative. In sections of spleen the AET treated cells adhered to the T cell areas only. Using untreated E adherence was also observed in the same areas, but considerably less. Thus the sensitivity of the E test with tissue sections is greatly enhanced using AET

TABLE 2. Haemadsorption to skin tissue sections of EA* sensitized by various amounts of antibodies

Skin	Agglutinating units of antibodies				
	2	1	1/2	1/4	1/8
Lesional	3+	2+	1–2+	1+	—
Normal	—	—	—	—	—

EA* = sheep erythrocytes sensitized with rabbit IgG antibody. See Materials and Methods for explanation of grading.



treated E. The results indicate that the majority of the dermal cellular infiltrate consists of T lymphocytes.

The degree of binding of the Fc γ receptors depended on the amount of A used for sensitization of E, i.e. stronger sensitization gave stronger reaction (Table 2). The results indicate the presence of macrophages and B lymphocytes in the infiltrate.

To differentiate the latter two cell populations complement receptor tests were performed. As shown in Figs 2 & 3 obviously more cells absorbed EAC3b than EAC3d. To some sections very few EAC3d indicator cells adhered, implying very few B lymphocytes.

DISCUSSION

Our results indicate that T lymphocytes are the predominant mononuclear cells in the dermal infiltrate in lichen planus. There are considerably less macrophages and only a few B lymphocytes. These results are in accordance with other reports. Tan *et al.* (1975) identified the inflammatory cells liberated from lesional skin of lichen planus with collagenase, and found that T lymphocytes predominated. Using an open technique Walker (1976) identified only small numbers of B lymphocytes and some macrophages by detection of their membrane receptors in frozen tissue sections. By exclusion he concluded that most of the mononuclear cells of the infiltrate were probably T lymphocytes. Accordingly, the infiltration of T lymphocytes in the dermis suggests that cell-mediated immune response may be of particular importance in the pathogenesis of lichen planus.

Till now, identification of T lymphocytes of skin tissue by E rosetting assay has usually been possible only in suspensions of mononuclear cells from lesional skin of various dermatoses (Edelson *et al.*, 1973; Tan *et al.*, 1975; Viac, Bustamante & Thivolet, 1977). However, this technique requires dense infiltration of lymphocytes in the tissue and does not give information about the topographic distribution of the different inflammatory cells. Stingl *et al.* (1977) have recently postulated that the E receptor can be detected only on living T lymphocytes and not on frozen cells *in situ*. This is contrary to our results and may be explained by differences in technique. They used an open method similar to most attempts to demonstrate T lymphocytes in tissue sections (Edelson *et al.*, 1973; Walker, 1976; Millard *et al.*, 1977). The closed chamber permits gentler handling of the tissue. This may be the main reason why we also detected T lymphocytes using untreated E, which is consistent with the results of Tønder *et al.* (1974) and Manthorpe *et al.* (1977). On the other hand, treatment with AET greatly enhanced the haemadsorption.

AET is a strong sulphhydryl reagent which alters erythrocyte membranes, resulting in enhanced binding of these cells to human T lymphocytes (Kaplan & Clark, 1974). The combination of AET treatment of erythrocytes and the closed chamber technique is a simple and reproducible test system for demonstration of T lymphocytes in skin tissue sections. The method presented is well suited also for small infiltrates, and the combined use of fixation and staining makes it possible to correlate the indicator cells to the cell infiltrate. However, some of the indicator cells will always be lost during fixation and staining. Therefore it is necessary to examine both living and stained preparations.

FIGURE 1. Skin section of lichen planus lesion showing adherence of AET treated E indicating T lymphocytes (stained with benzidine and H & E, $\times 225$).

FIGURE 2. Adherence of EAC3b indicating macrophages and B lymphocytes (stained with benzidine and H & E, $\times 225$).

FIGURE 3. Adherence of EAC3d indicating B lymphocytes (stained with benzidine and H & E, $\times 225$).

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REFERENCES

- ABRAMSON, N., GELFAND, E.W., JANDL, J.H. & ROSEN, F.S. (1970) The interaction between human monocytes and red cells. Specificity for IgG subclasses and IgG fragments. *Journal of Experimental Medicine*, **132**, 1207.
- ATKINSON, J.P. & FRANK, M.M. (1974) Studies on the *in vivo* effects of antibody. Interaction of IgM antibody and complement in the immune clearance and destruction of erythrocytes in man. *Journal of Clinical Investigation*, **54**, 339.
- CHRISTENSEN, B.E., JØNSSON, V., MATRE, R. & TØNDER, O. (1978) Traffic of T and B lymphocytes in the normal spleen. *Scandinavian Journal of Haematology*, **20**, 246.
- DICKLER, H.B. & KUNKEL, H.G. (1972) Interaction of aggregated γ -globulin with B lymphocytes. *Journal of Experimental Medicine*, **136**, 191.
- EDELSON, R.L., SMITH, R.W., FRANK, M.M. & GREEN, I. (1973) Identification of subpopulations of mononuclear cells in cutaneous infiltrates. I. Differentiation between B cells, T cells and histiocytes. *Journal of Investigative Dermatology*, **61**, 82.
- KAPLAN, M.E. & CLARK, C. (1974) An improved rosetting assay for detection of human T lymphocytes. *Journal of Immunological Methods*, **5**, 131.
- MANTHORPE, R., JØNSSON, V., CHRISTENSEN, B.E., HESSELVIK, M., EGEBERG, J. & VIDEBAEK, A. (1977) A case of T-cell lymphoma with Sézary cells in the blood and bone marrow accompanied by peripheral T and B lymphocytosis. *Scandinavian Journal of Haematology*, **18**, 449.
- MATRE, R. & TØNDER, O. (1976) Complement receptors in human renal glomeruli. *Scandinavian Journal of Immunology*, **5**, 437.
- MILDE, E.J. & TØNDER, O. (1968) Demonstration of rheumatoid factor in tissue by mixed agglutination with tissue sections. *Arthritis and Rheumatism*, **11**, 537.
- MILLARD, P.R., RABIN, B.S., WHITESIDE, T.L. & HUBBARD, J.D. (1977) The effects of tissue processing on markers for T and B cells from solid tissues. *American Journal of Clinical Pathology*, **67**, 230.
- REYNOLDS, H.Y., ATKINSON, J.P., NEWBALL, H.H. & FRANK, M.M. (1975) Receptors for immunoglobulin and complement on human alveolar macrophages. *Journal of Immunology*, **114**, 1813.
- ROSS, G.D. & POLLEY, M.J. (1975) Specificity of human lymphocyte complement receptors. *Journal of Experimental Medicine*, **141**, 1163.
- ROSS, G.D., POLLEY, M.J., RABELLINO, M.E. & GREY, H.M. (1973) Two different complement receptors on human lymphocytes. One specific for C3b and one specific for inactivator-cleaved C3b. *Journal of Experimental Medicine*, **138**, 798.
- RUDDY, S. & AUSTEN, K.F. (1971) C3b inactivator of man. II. Fragments produced by C3b inactivator cleavage of cell-bound or fluid phase C3b. *Journal of Immunology*, **107**, 742.
- STINGL, G., WOLFF, K., DIEM, E., BAUMGARTNER, G. & KNAPP, W. (1977) *In situ* identification of lymphoreticular cells in benign and malignant infiltrates by membrane receptor sites. *Journal of Investigative Dermatology*, **69**, 231.
- TAN, R.S.-H., BYROM, N.A. & HAYES, J.P. (1975) A method of liberating living cells from dermal infiltrate. Studies in skin reticuloses and lichen planus. *British Journal of Dermatology*, **93**, 271.
- TØNDER, O., MORSE, P.A., Jr & HUMPHREY, L.J. (1974) Similarities of Fc receptors in human malignant tissue and normal lymphoid tissue. *Journal of Immunology*, **113**, 1162.
- VIAC, J., BUSTAMANTE, R. & THIVOLET, J. (1977) Characterization of mononuclear cells in the inflammatory infiltrates of cutaneous tumours. *British Journal of Dermatology*, **97**, 1.
- WALKER, D.M. (1976) Identification of subpopulations of lymphocytes and macrophages in the infiltrate of lichen planus of skin and oral mucosa. *British Journal of Dermatology*, **94**, 529.
- WONG, L. & WILSON, J.D. (1975) The identification of Fc and C3 receptors on human neutrophils. *Journal of Immunological Methods*, **7**, 69.

Profile of epidermal metabolic activity in autosomal dominant ichthyosis and small bowel disorders

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SUMMARY

The *in vitro* incorporation of ^{14}C acetate by the epidermis has been studied in patients with autosomal dominant ichthyosis and in patients with a dry, itchy, slightly scaly skin associated with a disorder of the small bowel. Analysis of ^{14}C acetate containing lipid moieties by thin layer chromatography has indicated that there are both quantitative and qualitative differences in the uptake of ^{14}C acetate between autosomal dominant ichthyosis and normal. In particular an increased incorporation into the triglyceride and phosphatidyl choline fractions was noted. No such differences were apparent in those patients with disorders of the small bowel. In addition the *in vitro* incorporation of radioactively labelled thymidine, proline and histidine has been studied in these patients. In both groups of patients the rate of incorporation of tritiated thymidine and histidine into epidermal macromolecules was found not to differ significantly from normal. On the other hand the rate of incorporation of tritiated proline was increased in both groups of patients.

The evidence for the involvement of lipids in disordered keratinization is well documented. Drugs such as triparanol (Achor, Winkelmann & Perry, 1961) and nicotinic acid (Ruiter & Meyler, 1960) which interfere with cholesterol biosynthesis have been responsible for a dry scaly skin condition resembling autosomal dominant ichthyosis. Increased ratios of sterol to sterol esters have been reported in psoriasis (Wheatley & Farber, 1961) as has altered lipid biosynthesis (Cooper, McGrath & Shuster, 1976). In Refsum's disease there is an accumulation of phytanic acid in the tissues together with an apparent substitution of this fatty acid for linoleic and arachidonic acid in the phospholipid moieties (Malmendier *et al.*, 1974; Dykes *et al.*, 1978). Essential fatty acid deficiency also causes an ichthyotic state both clinically and experimentally (Press *et al.*, 1974; Prottey, 1976). A dry, itchy, slightly scaling skin commonly accompanies severe small bowel disease and is seen in patients with gluten enteropathy and others with Crohn's disease and ulcerative colitis, whether there is mal-absorption or not.

In order to help define the relationship between lipids and disordered desquamation we have

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studied the pattern of *in vitro* incorporation of ^{14}C acetate into epidermal lipids in patients with autosomal dominant ichthyosis and patients with certain disorders of the small bowel. In addition, as an indication of general metabolic activity, the *in vitro* rate of incorporation of tritiated thymidine, proline and histidine into epidermal macromolecules has been measured.

PATIENTS AND METHODS

Patients

In the first part of this study, skin samples were obtained from the thighs of ten individuals with normal skin who had given their informed consent (4 male, 6 female, mean age 47), nine patients with autosomal dominant ichthyosis (5 male, 4 female, mean age 41) as defined by Frost (1973) and ten patients with various disorders of the small bowel. The diagnoses of the latter group of patients, which we have called the malabsorptive group, are given in Table 1. They all had a dry, slightly scaly

TABLE 1. Clinical details of patients with small bowel disorders

Patient number	Age	Sex	Diagnosis
1*	63	M	Coeliac disease
2	46	M	Coeliac disease
3	42	M	Coeliac disease
4	54	M	Crohn's disease
5	56	F	Crohn's disease with a history of multiple intestinal resections
6	69	M	Proctocolitis
7†	56	F	Colitis
8	45	F	Ileo jejunal bypass for obesity 1 year previously
9	39	F	Ileo jejunal bypass for obesity 4 years previously
10	53	M	Partial gastrectomy for duodenal ulcer 10 years previously
11*	65	M	Partial gastrectomy for duodenal ulcer 4 years previously

* Not used in ^{14}C acetate study.

† Not used in tritiated precursor study.

and pruritic skin sufficient to be noted by both patient and a gastroenterologist (P.S.). Gas-liquid chromatographic analysis of the plasma fatty acids of these patients failed to demonstrate the presence of 5, 8, 11 eicosatrienoic acid, indicating that none of this group was suffering from overt essential fatty acid deficiency.

In the second part of this study skin samples were obtained from the thighs of six normal individuals with normal skin who had given their informed consent (4 males, 2 females, mean age 44), seven patients with autosomal dominant ichthyosis (4 males, 3 females, mean age 42) and nine patients with various disorders of the small bowel (See Table 1).

In vitro incorporation of ^3H thymidine, proline and histidine

Thin skin sheets were obtained using a Castroviejo keratome set at 0.4 mm, and cut into rectangles approximately 50 mm square, before incubation *in vitro* with tritium labelled thymidine, proline or histidine. (Such sheets contain only small amounts of contaminating dermis and no sebaceous glands). Specimens were incubated for 4 h at 37°C in Eagle's MEM containing 1 $\mu\text{Ci}/\text{ml}$ of either thymidine or proline or histidine and processed for scintillation counting as described previously

(Marks, Fukui & Halprin, 1971). Three specimens from each patient were incubated with each precursor and the results are expressed as mean d.p.m. per square mm skin surface area per hour incubation. In some cases an additional skin specimen was incubated with tritiated proline and fixed in phosphate buffered formalin. These specimens were processed by routine histological methods and prepared for autoradiography as described by Shahrada & Marks (1976). Using high power magnification the number of autoradiographic grains per cell was determined microscopically in randomly selected areas of the dermis. At least 5 areas from each specimen were counted and the results for each specimen expressed as the mean number of grains per dermal cell. The number of contaminating dermal cells per microscope field was also determined.

In vitro incorporation of ^{14}C acetate

Skin specimens obtained with a keratome were incubated at 37°C in Eagle's MEM containing 10% human serum and $25\ \mu\text{Ci/ml}$ of ^{14}C labelled acetate ($1-^{14}\text{C}$ acetic acid, sodium salt. Specific activity = $60\ \text{mCi/mmol}$). Two specimens from each patient were incubated. After incubation the skin samples were rinsed briefly in saline and stored at -20°C until extracted and analysed by thin layer chromatography. An incubation period of 4 h was chosen as it has been shown that the incorporation of acetate is linear over this time period (Wheatley, 1974). Such short term culture also minimizes the risk of artefacts due to cell death and possible reutilization of precursor material after lipolysis.

Lipid extraction and analysis

Skin specimens were homogenized in distilled water and precipitated with 10% perchloric acid. After washing with 2% perchloric acid and distilled water the precipitates were extracted twice with chloroform-methanol (2:1 v/v). Extracts were pooled and after concentration at 40°C using a stream of nitrogen gas, analysed by thin layer chromatography on precoated thin layer plates ($20 \times 20\ \text{cm}$) of silica gel 60 (Merck, A.G. Darmstadt, Germany) which had previously been activated at 100°C for 30 min. Lipid analysis was performed as described by Summerly & Woodbury (1971). Plates were developed firstly in hexane-benzene (1:1 v/v) and secondly in the same dimension with petroleum ether (b.p. $60-80^\circ\text{C}$)-diethyl ether-acetic acid (80:20:1, by vol).

Phospholipids were analysed using the single development system of chloroform-methanol-acetic acid-water (50:30:8:4, by vol), described by Skipski, Peterson & Barclay (1964). This system was chosen because of its simplicity and its ability to separate those phospholipids which are reported as being the most prevalent in the human epidermis (Gerstein, 1963). After development the radioactive bands were located using a Panax thin layer scanner (Panax Equipment Ltd., Redhill, Surrey) and identified by co-chromatography with reference compounds obtained from the Sigma Chemical Co., Ltd., London. Those areas corresponding to the bands were then removed from the plate and the radioactivity eluted and estimated by scintillation counting. Results for each band are expressed as d.p.m. per mm^2 skin surface per hour incubation.

RESULTS

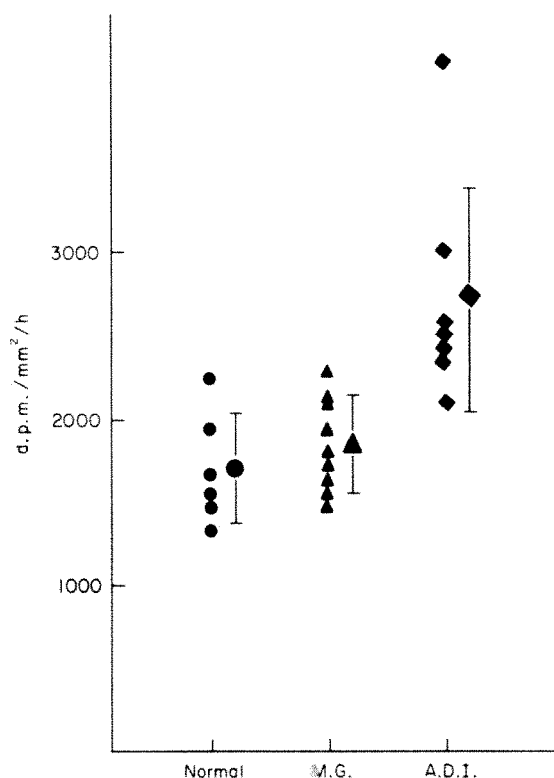
In vitro incorporation of ^3H thymidine, proline and histidine

The *in vitro* rates of incorporation of the tritiated precursors into macromolecules are given in Table 2. Whilst the thymidine and histidine values did not differ significantly from normal in either group, proline uptake was increased in both the malabsorptive group ($P < 0.05$) and in the ichthyosis group ($P < 0.01$).

There was no preferential uptake of proline by those dermal cells present in the ichthyosis specimens. The mean value ($\pm$ standard deviation) for the number of autoradiographic grains per dermal

TABLE 2. Incorporation of radioactive precursors into epidermal macromolecules

	DPM/mm ² /h $\pm$ s. d.		
	Thymidine	Proline	Histidine
Normal	98 $\pm$ 32 (n=10)	223 $\pm$ 53 (n=9)	89 $\pm$ 37 (n=9)
Malabsorptive group	101 $\pm$ 51 (n=10)	308 $\pm$ 94*	105 $\pm$ 42 (n=9)
Autosomal dominant ichthyosis	131 $\pm$ 46 (n=9)	469 $\pm$ 169†	145 $\pm$ 75 (n=9)

* Significantly different from normal $0.05 > P > 0.02$ (Students 't'-test).† Significantly different from normal $0.01 > P > 0.001$ (Students 't'-test).FIGURE 1. Total radioactivities recovered from the thin layer chromatography plates after analysis of ¹⁴C acetate labelled lipid extracts. The individual values for the normals, the malabsorptive group (MG) and the autosomal dominant ichthyosis group (ADI) are shown together with the mean $\pm$ standard deviation for each group.

cell in the ichthyosis group was 2.24 ± 0.98 ($n=5$). This did not differ significantly from the normal values, 2.88 ± 1.63 ($n=5$). Likewise the number of contaminating dermal cells per microscope field in the ichthyotic group (55 ± 14 , $n=8$) did not differ significantly from normal values (50 ± 16 , $n=6$).

Incorporation of ^{14}C acetate into total recoverable lipids

The total radioactivities recoverable from the chromatography plates for the three groups of patients are given in Fig. 1. A quantitative difference is apparent, there being 62% more radioactivity recoverable in the ichthyosis group than in the normals ($P < 0.01$). A small, but statistically insignificant increase was observed in the malabsorptive group.

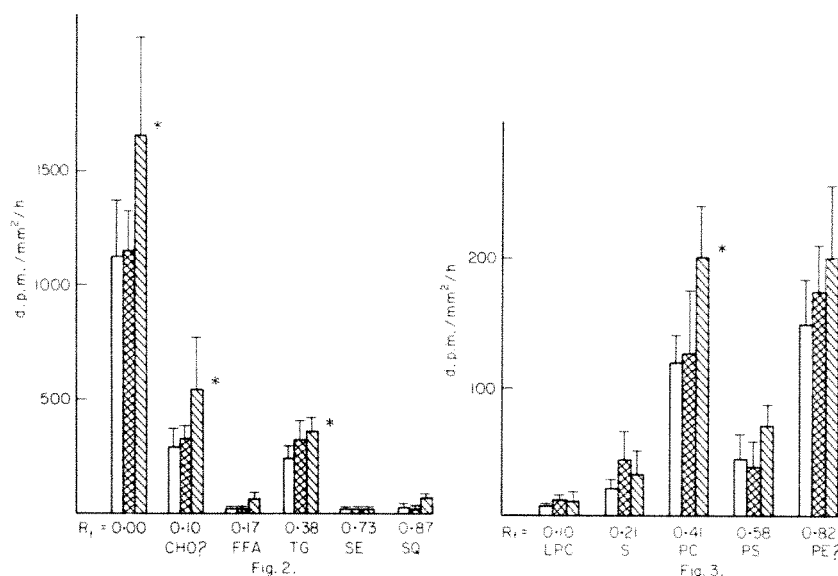


FIGURE 2. Pattern of *in vitro* incorporation of ^{14}C acetate into epidermal lipids in normal individuals (open columns), the malabsorptive group (MG) (cross-hatched columns) and autosomal dominant ichthyosis (ADI) (hatched columns). The mean $\pm$ standard deviation for each of the six bands identified after development with the solvent system of Summerly & Woodbury (1971) are shown. Those bands where the values for the ichthyosis group are significantly different from normal ($P < 0.05$) are indicated by an asterisk. The R_f values (mean $\pm$ standard deviation) of the reference compounds used were: cholesterol $R_f = 0.06 \pm 0.01$, oleic acid, $R_f = 0.17 \pm 0.03$, triolein, $R_f = 0.39 \pm 0.04$, cholesterol oleate, $R_f = 0.75 \pm 0.02$, squalene $R_f = 0.85 \pm 0.021$. Abbreviations: CHO = cholesterol; FFA = free fatty acids; TG = triglycerides; SE = sterol esters; SQ = squalene.

FIGURE 3. Pattern of *in vitro* incorporation of ^{14}C acetate into epidermal phospholipids in normal individuals (open columns), the malabsorptive groups (MG) (cross-hatched columns) and autosomal dominant ichthyosis (ADI) (hatched columns). The mean $\pm$ standard deviation for each of the five bands identified after development with the solvent system of Skipski *et al.* (1964) are shown. The value for the band corresponding to phosphatidyl choline in the ichthyosis groups was significantly different from normal ($P < 0.05$) and is indicated by an asterisk. The R_f values (mean $\pm$ standard deviation) of the reference compounds used were: Lyso-phosphatidyl choline $R_f = 0.10 \pm 0.02$, sphingomyelin $R_f = 0.24 \pm 0.07$, phosphatidyl choline $R_f = 0.42 \pm 0.03$, phosphatidyl serine $R_f = 0.57 \pm 0.06$, phosphatidyl ethanolamine $R_f = 0.87 \pm 0.06$, phosphatidyl inositol $R_f = 0.56 \pm 0.06$. Abbreviations: LPC = lyso-phosphatidyl choline; S = sphingomyelin; PC = phosphatidyl choline; PS = phosphatidyl serine; PE = phosphatidyl ethanolamine.

Incorporation of ^{14}C acetate in individual lipid moieties

The radioactivities recoverable from each of the six bands detectable after development with the solvent system of Summerly & Woodbury (1971) are compared in Fig. 2. No significant differences are apparent between the malabsorptive group and normal individuals. However, in certain of the fractions, those bands at the origin, the cholesterol and triglyceride positions, the values for the ichthyosis group were significantly increased compared to normal.

Apart from material running with the solvent front, five radioactive bands were detected using the solvent system of Skipski, Peterson & Barclay (1964) (Fig. 3). Again no significant differences were detected between malabsorptive group and normals. This is again contrasted by the ichthyosis group where the radioactivity recoverable in the phosphatidyl choline fraction was significantly increased compared to normal.

DISCUSSION

It would appear that an increased *in vitro* incorporation of proline is a common feature of these two groups of patients. In the ichthyosis group at least this appears to be due to epidermal cells as there is no difference in the number of contaminating dermal cells, and autoradiographically these cells do not show any preferential uptake of proline. Whether this observation is related to the disordered keratinization apparent in this group is uncertain. An autoradiographic study of the uptake of tritium labelled amino acids by the various layers of the rat epidermis did not reveal any preferential labelling of a particular cell layer by proline (Fukuyama & Epstein, 1966). However, it has been reported that both keratohyalin granules and the membrane proteins of the horny layer have a high proline content (Matoltsy & Matoltsy, 1970). A distinguishing feature of autosomal dominant ichthyosis at an ultra-structural level is an altered keratohyalin granule structure (Anton-Lamprecht & Hofbauer, 1972) and it may well be that these changes and the altered proline metabolism are related.

The *in vitro* studies involving incorporation of ^{14}C acetate have indicated that there are both quantitative and qualitative differences in ^{14}C acetate uptake in ichthyosis when compared to normals. Changes in precursor utilization have also been observed in ichthyosis in studies with ^{14}C labelled glucose (Cooper, McGibbon & Shuster, 1978). The quantitative differences observed do not appear to be related to increased cell turnover. Fisher & Wells (1968) and Frost (1973) have reported normal epidermal cell kinetics in autosomal dominant ichthyosis and our own studies are in agreement (Marks & Dykes, 1978). Our value for the *in vitro* rate of incorporation of tritiated thymidine (Table 2) is also not significantly different from normal. The quantitative difference is not explicable on the basis of increased epidermal volume due to a greater number of cells or increased individual cell size. Although it has been reported that the epidermal thickness is increased by approximately 20% in autosomal dominant ichthyosis (Fraki, Kuokkanen & Hopsu-Havu, 1973), measurements in this group of patients failed to demonstrate a statistically significant increase relative to normal (Dykes & Marks, unpublished observations). The question as to whether overall lipid metabolism is increased or whether there is a net increase in lipid synthesis per se requires further study. The significance of the qualitative changes seen are also uncertain. For example the factors which would lead to increased uptake of ^{14}C acetate into the phosphatidyl choline fraction are obscure. Such incorporation is predominantly related to fatty acid synthesis and turnover which operates independently of and at a faster rate than the phospholipid moiety as a whole. As such the qualitative difference observed may indicate an abnormality of membrane lipid biosynthesis which, in the case of the plasma membrane, may be sufficient to cause abnormal desquamation. The failure to find significant differences in incorporation between the normal and malabsorptive groups is perhaps surprising. Whilst overt essential fatty acid deficiency is relatively uncommon in this type of patient, changes in the plasma

lipid composition have been noted (Shimoyama *et al.*, 1973). This is especially true in those patients who have undergone intestinal resection, although the changes are not necessarily related to the site or extent of the resection (Collins *et al.*, 1971). The relationship between the plasma and epidermal fatty acid composition, and therefore metabolism, is not clear. However, they are undoubtedly related (Prottey, 1976). In general our malabsorptive group were mildly affected and further work on this group of patients, particularly those with intestinal resection, may help elucidate this matter.

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REFERENCES

- ACHOR, R.W.P., WINKELMANN, R.K. & PERRY, H.O. (1961) Cutaneous side effects from use of Triparanol (Mer 29): Preliminary data on ichthyosis and loss of hair. *Proceedings of the Mayo Clinic*, **36**, 217.
- ANTON-LAMPRECHT, I. & HOFBAUER, M. (1972) Ultrastructural distinction of autosomal dominant ichthyosis vulgaris and x-linked recessive ichthyosis. *Human Genetics*, **15**, 261.
- COLLINS, F.D., SINCLAIR, A.J., ROYLE, J.P., COATS, D.A., MAYNARD, A.T. & LEONARD, R.F. (1971) Plasma lipids in human linoleic acid deficiency. *Nutrition and Metabolism*, **13**, 150.
- COOPER, M.F., MCGRATH, H. & SHUSTER, S. (1976) Epidermal lipid metabolism in psoriasis and lichen simplex. *British Journal of Dermatology*, **94**, 369.
- COOPER, M.F., MCGIBBON, D. & SHUSTER, S. (1978) Epidermal lipogenesis in ichthyosis. In: *The Ichthyoses* (Ed. by R. Marks and P.J. Dykes). MTP Press, Lancaster.
- DYKES, P.J., MARKS, R., DAVIES, M.G. & REYNOLDS, D.J. (1978) Epidermal metabolism in hereditary atactic polyneuritis (Refsum's disease). *Journal of Investigative Dermatology*, in press.
- FISHER, L.B. & WELLS, G.C. (1968) The mitotic rate and duration in lesions of psoriasis and ichthyosis. *British Journal of Dermatology*, **80**, 235.
- FUKUYAMA, K. & EPSTEIN, W.L. (1966) Epidermal keratinisation: localization of isotopically labelled amino acids. *Journal of Investigative Dermatology*, **47**, 551.
- FRANK, J.E., KUOKKANEN, K. & HOPUSU-HAVU, V.K. (1973) Morphometric analysis of the dominant and sex-linked forms of ichthyosis vulgaris. *Acta dermato-venereologica*, **53**, 299.
- FROST, P. (1973) Ichthyosiform dermatoses. *Journal of Investigative Dermatology*, **60**, 541.
- GERSTEIN, W. (1963) The phospholipids of normal and psoriatic skin. *Journal of Investigative Dermatology*, **40**, 105.
- MALMENDIER, C.L., JONNIAUX, G., VOET, W. & VAN DEN BERGEN, C.J. (1974) Fatty acid composition of tissues in Refsum's disease. (Hereditary Atactic Polyneuritis). Estimation of total phytanic acid accumulation. *Biomedicine*, **20**, 398.
- MATOLTSY, A.G. & MATOLTSY, M. (1970) The chemical nature of the keratohyalin granule of the epidermis. *Journal of Cell Biology*, **47**, 593.
- MARKS, R. & DYKES, P.J. (1978) Epidermal growth characteristics in the ichthyotic disorders. In: *The Ichthyoses* (Ed. by R. Marks and P.J. Dykes). MTP Press, Lancaster.
- MARKS, R., FUKUI, K. & HALPRIN, K. (1971) The application of an *in vitro* technique to the study of epidermal replication and metabolism. *British Journal of Dermatology*, **84**, 453.
- PRESS, M., KIKUCHI, H., SHIMOYAMA, T. & THOMPSON, G.R. (1974) Diagnosis and treatment of essential fatty acid deficiency in man. *British Medical Journal*, **ii**, 247.
- PROTTEY, C. (1976) Essential fatty acids and the skin. *British Journal of Dermatology*, **94**, 579.
- RICHTERICH, R.P., VAN MECHELEN, P. & ROSSI, E. (1965) Refsum's disease (Hereditary Atactic Polyneuritis). An inborn error of lipid metabolism with storage of 3, 7, 11, 15 tetramethyl hexadecanoic acid. *American Journal of Medicine*, **39**, 320.
- RUITER, M. & MEYLER, L. (1960) Skin changes after therapeutic administration of nicotinic acid in large doses. *Dermatologica*, **120**, 139.
- SHAHRAH, P. & MARKS, R. (1976) Hair follicle kinetics in psoriasis. *British Journal of Dermatology*, **94**, 7.
- SHIMOYAMA, T., KIKUCHI, H., PRESS, M. & THOMPSON, G.R. (1973) Fatty acid composition of plasma lipoproteins in control subjects and in patients with malabsorption. *Gut*, **14**, 716.

- SKIPSKI, V.P., PETERSON, R.F. & BARCLAY, M. (1964) Quantitative analysis of phospholipids by thin layer chromatography. *Biochemical Journal*, **90**, 374.
- SUMMERLY, R. & WOODBURY, S. (1971) The *in vitro* incorporation of ^{14}C acetate into the isolated sebaceous gland and appendage free epidermis of human skin. *British Journal of Dermatology*, **85**, 424.
- WHEATLEY, V.R. (1974) Cutaneous lipogenesis. Major pathways of carbon flow and possible interrelationships between the epidermis and sebaceous glands. *Journal of Investigative Dermatology*, **62**, 245.
- WHEATLEY, V.R. & FARBER, E.M. (1961) Studies on the chemical composition of psoriatic scales. *Journal of Investigative Dermatology*, **36**, 199.

Corticosteroid effect on epidermal cell size

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SUMMARY

Changes in the epidermis following application of three corticosteroids, betamethasone 17-valerate, hydrocortisone 17-butyrate, and hydrocortisone have been studied histometrically in human volunteers. The reduction in epidermal thickness observed correlated significantly with a reduction in size of the viable epidermal cells. There was no significant reduction in the number of cells constituting the viable epidermis. These findings indicate that thinning of the epidermis is a function of cell size rather than cell number. The epidermal changes developed quickly and were rapidly reversible. It is suggested that measurement of cell size may be an early and sensitive index of atrophogenicity induced by topical corticosteroids.

0.1% Hydrocortisone 17-butyrate and 0.1% betamethasone 17-valerate showed equivalent potency in causing epidermal thinning and reduction in cell size. Reduction in cell size paralleled increasing concentrations of betamethasone 17-valerate, indicating a positive dose-effect relationship.

Skin atrophy is a well known side effect of topical corticosteroids and possible factors in its pathogenesis have been reviewed (Marks, 1976). Both the epidermis and dermis are affected by applications of topical corticosteroids. Marks (1976) has summarized the methods available for quantifying the degree of skin atrophy found. These methods, however, give limited information as to the changes occurring within the epidermis. The epidermis contributes very little to the total skin thickness and it is therefore unlikely that epidermal thinning plays much of a role in the appearances of skin atrophy. However, its existence is important because quantitative changes in epidermal structure may give insight into the nature of the atrophic effects in general. Reduction in epidermal cell size has been observed previously following application of corticosteroids (Winter & Burton, 1976; Wilson Jones, 1976), but has not been quantified or systematically studied.

Histometric techniques may be employed to assess epidermal thickness and epidermal cell size (Holt & Marks, 1976). In this study we have measured thickness and cell size following the application of three corticosteroids (betamethasone 17-valerate, hydrocortisone 17-butyrate and hydrocortisone), and a bland preparation (white soft paraffin). In addition we have attempted to determine the time course of these epidermal changes following application of 0.1% betamethasone 17-valerate

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ointment and investigated whether a positive dose-effect relationship existed between betamethasone 17-valerate and these epidermal measurements.

MATERIALS AND METHODS

The subjects studied were informed healthy male adult volunteers.

Experiment 1

Five subjects aged 35–77 years (mean 61 years) were studied. In all subjects white soft paraffin (WSP), 1% hydrocortisone, 0.1% hydrocortisone 17-butyrate and 0.1% betamethasone 17-valerate ointments were applied thinly without occlusion twice daily to four test sites on the upper arms for 14 days. Then 4 mm punch biopsies were taken from each test site.

Experiment 2

Six subjects aged 20–56 (mean 34 years) were studied. 0.1% betamethasone 17-valerate ointment was thinly applied twice daily without occlusion to a test site on the lateral aspect of the thigh for 28 days. In all instances 4 mm punch biopsies were taken immediately before the commencement of the study (day 0) and on days, 4, 28 and 42 (4 subjects), days 2, 28 and 56 (1 subject) and days 7, 28 and 56 (1 subject) and days 7, 28 and 56 (1 subject).

Experiment 3

Four subjects aged 20–55 years (mean 40 years) were studied. Preparations of 0.01, 0.025 and 0.1% betamethasone 17-valerate ointment and WSP were applied thinly without occlusion twice daily to four test sites on the upper arms for 28 days. Then 4 mm punch biopsies were taken from each test site.

EPIDERMAL DIMENSIONS

Skin biopsy specimens were fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned and stained with haematoxylin and eosin. The number of cell layers forming the viable epidermal compartment was determined by direct counting of nuclei from the basal layer up to the granular cell layer along an axis perpendicular to the skin surface at 20 random points and expressed as the mean epidermal cell number (MECN). The mean epidermal thickness (MET) was determined by measuring this same distance with a calibrated eyepiece graticule.

The rete pattern of the epidermis was measured by calculating the ratio of the length of the basal layer to the length of the granular cell layer (B/G ratio) using a microscope drawing tube projection technique. The size of the keratinocytes was expressed as the mean keratinocyte height (MKH). This was calculated by dividing the MET by the MECN.

RESULTS

Experiment 1

The results are shown in Table 1. The MKH was reduced ($P < 0.05$) in skin treated with 0.1% hydrocortisone 17 butyrate and 0.1% betamethasone 17-valerate by 30 and 28% respectively, and the MET was reduced by 27 and 29% respectively when compared to the control values. There was a

TABLE 1. Comparison of epidermal dimensions following twice daily applications of 1% hydrocortisone, 0.1% hydrocortisone 17-butyrate, 0.1% betamethasone 17-valerate ointments and white soft paraffin (control). Mean $\pm$ s.e. of mean

Application	B/G ratio	MKH (μ m)	MECN	MET (μ m)
White soft paraffin	1.08 $\pm$ 0.04	10.93 $\pm$ 0.2	3.22 $\pm$ 0.18	35.2 $\pm$ 2.0
1% Hydrocortisone ointment	0.96 $\pm$ 0.02	9.62 $\pm$ 0.76	3.48 $\pm$ 0.1	33.53 $\pm$ 3.08
0.1% Hydrocortisone 17-butyrate	0.99 $\pm$ 0.03	7.98 $\pm$ 0.16	3.07 $\pm$ 0.28	24.45 $\pm$ 2.1
0.1% Betamethasone 17-valerate ointment	0.98 $\pm$ 0.01	7.71 $\pm$ 0.32	3.31 $\pm$ 0.4	25.39 $\pm$ 3.15

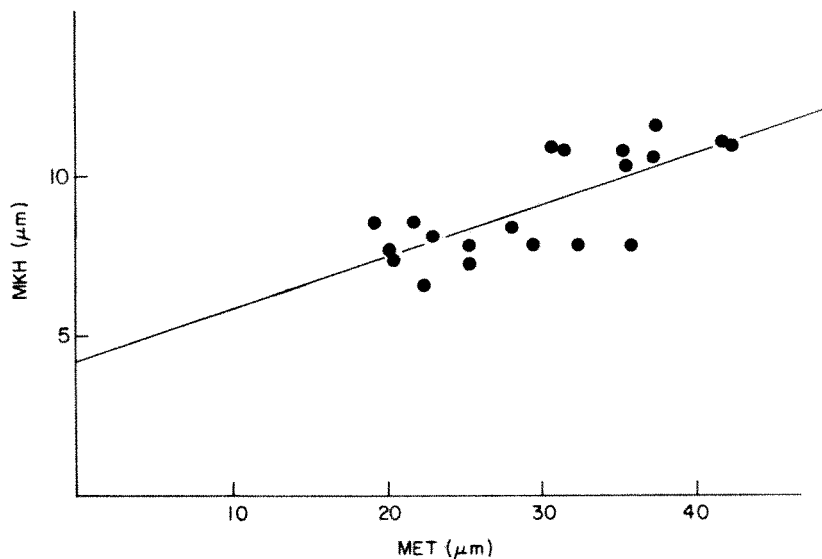


FIGURE 1. Correlation between mean keratinocyte height (MKH) and mean epidermal thickness (MET). $r=0.75$, $0.05 > P > 0.01$, $y=0.17x + 4.17$.

positive correlation ($r=0.75$, $P<0.05$) between the MKH and MET (Fig. 1). In contrast, no significant reduction in the MECN ($P>0.05$) was observed in any of the specimens obtained from the corticosteroid treated sites. The MKH was reduced ($P>0.05$) by only 12% of the control value in skin treated with 1% hydrocortisone ointment.

Experiment 2 (Table 2)

A reduction in the MET and MKH was observed as early as the 4th day of application with 0.1%

TABLE 2. Epidermal changes in relationship to time. 0.1% betamethasone 17-valerate ointment was applied twice daily for 28 days.
Mean $\pm$ s.e. of mean

Time	MKH (μ m)	MECN	MET (μ m)
Day 0	12.77 $\pm$ 0.50	3.30 $\pm$ 0.10	42.24 $\pm$ 3.05
Day 4	11.81 $\pm$ 0.86	3.26 $\pm$ 0.17	38.66 $\pm$ 3.76
Day 28	10.22 $\pm$ 0.56	3.22 $\pm$ 0.16	32.87 $\pm$ 1.86
Day 42	12.60 $\pm$ 0.53	3.06 $\pm$ 0.43	38.66 $\pm$ 2.05

betamethasone 17-valerate. After 28 days the MKH and MET were reduced ($P < 0.05$) by 20 and 27% of the control values respectively. There was no significant reduction in the value of the MECN. Fourteen days after stopping applications, the MKH and MET had returned to the pre-treatment values.

Experiment 3 (Table 3)

Higher concentrations of betamethasone 17-valerate induced greater reductions in epidermal thickness and cell size than did lower concentrations. 0.1% betamethasone 17-valerate reduced the MET and MKH by 22% ($P < 0.05$) and 13% ($P < 0.05$) of the control values respectively.

TABLE 3. The dose-effect relationship between betamethasone 17-valerate and epidermal dimensions. Means $\pm$ s.e. of mean. Betamethasone 17-valerate ointment was applied in the concentrations indicated to the test sites twice daily for 28 days. White soft paraffin served as a control

Application	MKH (μ m)	MECN	MET (μ m)
White soft paraffin	12.76 $\pm$ 0.47	3.37 $\pm$ 0.17	42.42 $\pm$ 3.78
0.01% Beta-metasone-17 valerate	11.53 $\pm$ 1.07	3.41 $\pm$ 0.06	39.51 $\pm$ 3.84
0.025% Beta-methasone-17 valerate	11.39 $\pm$ 0.99	3.0 $\pm$ 0.09	34.31 $\pm$ 3.47
0.1% Beta-methasone-17 valerate	11.10 $\pm$ 0.94	3.0 $\pm$ 0.05	33.22 $\pm$ 2.35

DISCUSSION

The observed changes in epidermal measurements seen following the application of corticosteroids confirm that the epidermis is influenced by the local action of corticosteroids, and indicate that these changes take place in a short period of time, and are rapidly reversible.

The significant correlation seen between epidermal thickness and keratinocyte size indicates that

thinning of the epidermis is a function of cell size rather than cell number. We suggest that measurement of cell size may be an early and sensitive index of atrophogenicity from topical corticosteroids.

It is well established that topical corticosteroids have an antimitotic effect on epidermal cells (Fisher & Maibach, 1971; Marks, Pongsehirun & Saylan, 1973), and that this may contribute to the corticosteroid-induced atrophy of the epidermis. However, reduced cell size may be more directly related to reduced macromolecular synthesis by keratinocytes (Marks *et al.*, 1971). It may be relevant that alterations in ribosomes and endoplasmic reticulum within keratinocytes have been described in steroid-induced atrophic skin (Stefanovic, 1976).

0.1% hydrocortisone 17-butyrate and 0.1% betamethasone 17-valerate showed equivalent potency in causing epidermal thinning and reduction in cell size. Kirby & Munro (1976) have shown that 0.1% hydrocortisone 17-butyrate induced greater thinning of mouse skin than 0.1% betamethasone 17-valerate. This hydrocortisone ester has also been shown to produce greater dermal atrophy than betamethasone 17-valerate (Dykes & Marks, 1977).

The gradual reduction seen in cell size with increasing concentrations of betamethasone 17-valerate suggests that this alteration in keratinocyte size is not an all-or-none phenomenon and indicates a positive dose response effect.

REFERENCES

- DYKES, P.J. & MARKS, R. (1977) Measurement of skin thickness. A comparison of two *in vivo* techniques with a conventional histometric method. *Journal of Investigative Dermatology*, **69**, 275.
- FISHER, L.B. & MAIBACH, H.I. (1971) The effect of corticosteroids on human epidermal mitotic activity. *Archives of Dermatology*, **103**, 39.
- HOLT, P.J.A. & MARKS, R. (1976) Epidermal architecture, growth and metabolism in acromegaly. *British Medical Journal*, **i**, 496.
- KIRBY, J.D. & MUNRO, D.D. (1976) Steroid-induced atrophy in an animal and human model. *British Journal of Dermatology*, **94**, Suppl. **12**, 111.
- MARKS, R. (1976) Methods for the assessment of skin atrophogenicity of topical corticosteroids. *Dermatologica*, **152**, Suppl. **1**, 117.
- MARKS, R., HALPRIN, K., FUKUI, J. & GRAFF, D. (1971) Topically applied triamcinolone and macromolecular synthesis by human epidermis. *Journal of Investigative Dermatology*, **56**, 470.
- MARKS, R., PONGSEHIRUN, D. & SAYLAN, R. (1973) A method for the assay of topical corticosteroids. *British Journal of Dermatology*, **88**, 69.
- STEFANOVIC, D.V. (1976) Corticosteroid-induced atrophy of the epidermis. In: *Mechanisms of Topical Corticosteroid Activity* (Ed. by L.C. Wilson and R. Marks), p. 97. Churchill Livingstone, Edinburgh.
- WILSON JONES, E. (1976) Steroid atrophy—a histological appraisal. *Dermatologica*, **152**, Suppl. **1**, 107.
- WINTER, G.D. & BURTON, J.L. (1976) Experimentally induced steroid atrophy in the domestic pig and man. *British Journal of Dermatology*, **94**, Suppl. **12**, 107.

Preparation of thick tissue sections using cellophane tape

AN IMPROVED METHOD

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It has been quite difficult to get good thick tissue sections using the techniques available to date, so Inaba & Yamamura (1975) developed a cellophane tape method which enabled them to study tissue morphology easily.

However, complete removal of paraffin was hard to attain with the method as it stood, so improvements were introduced to remedy this deficiency and allow better staining. These refinements form the substance of this report.

METHOD

Specimen sectioning

Cellophane tape is attached to a paraffin-embedded specimen (Fig. 1). The latter is then cut to the required thickness (200–500 µm) with a microtome.

Application of sectioned specimen

The thick-sectioned specimen is pressed onto a glass slide as follows:

Egg albumen-glycerine is spread thinly on the centre of the slide to prevent the specimen from peeling off, and then, with the cellophane tape uppermost, it is firmly pressed with the palm onto the slide (Fig. 2), and subsequently placed in a paraffin distending apparatus at 50–60°C and dried for some time.

Removal of cellophane tape

Soaking the specimen in xylol suffices, but recently, in order to shorten the time, the specimen is put instead in a dry heat sterilization apparatus (60°C) and warmed for 2–3 min. Once the stickiness is reduced, the cellophane tape can be removed.

Removal of paraffin

Again, simply soaking in the xylol is sufficient, but for speed and completeness the xylol can be care-

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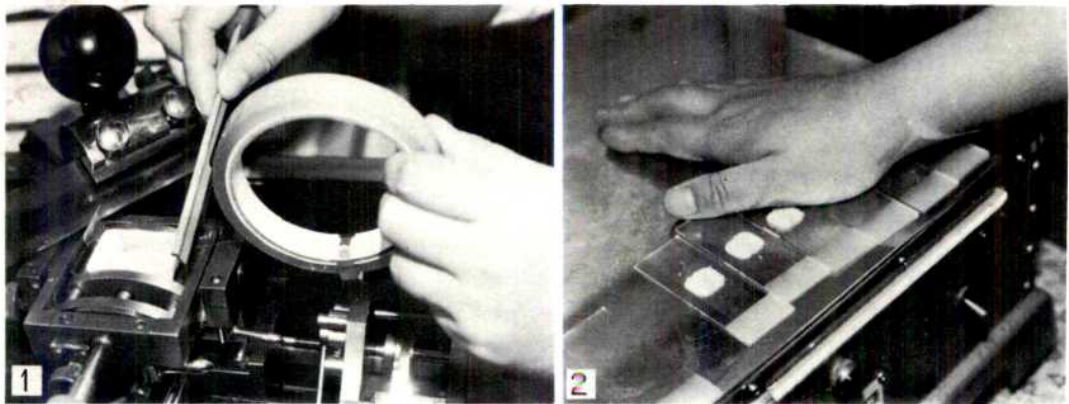


FIGURE 1. Cellophane tape is attached to a paraffin-embedded specimen.

FIGURE 2. The specimen, with cellophane side uppermost, is pressed firmly onto the slide.

fully warmed in a hot bath at 50–60°C for about 5 min to allow the paraffin to come out easily. To ensure that its removal is complete, however, the specimen is put in fresh xylol for about 30 min.

Staining method

Different staining methods can be used, but as thick sectioned specimens have a tendency to stain deeply, the staining time for H & E is shortened; to 3 min for the haematoxylin and 5 min for the eosin. After staining, the specimen should be thoroughly rinsed with water for about 30 min.

Mounting

For mounting, balsam is used.

OBSERVATIONS

It was possible to study stereo-histological morphology using serial thick sections prepared by this method. The following are a few examples.

(1) Visualization of the apocrine gland on its own (Fig. 3)

Axillary malodour can be reduced by electrocoagulation. To verify that the apocrine gland can remain after removal of the hair, our method can be used to visualize the area.

Thus, in Fig. 3, it can be seen that:

- (a) The apocrine gland secretory duct is enlarged.
- (b) The axillary hair and its accompanying sebaceous gland have vanished, leaving just the apocrine gland.

These findings were demonstrated in more than ten such cases, and they suggest that during depilation, hair regrowth no longer occurs once the sebaceous gland has been destroyed.

(2) Theory of hair development

The relationship between the sebaceous gland and the growth and regrowth of hair has cast doubt on the currently accepted theory of hair growth. Hair starts growing from the secretory portion of the

sebaceous gland, and it has been observed that in hair formation the hair bulb is completed last. The following showed that young hair does grow during the hair germ stage.

(a) Young axillary hair can be seen to grow from the secretory portion of the sebaceous gland if the latter is left intact after the subcutaneous tissue has been removed by a subcutaneous shaver (Inaba, 1977) (Figs 4, 5).

Moreover, axillary hair is seen to regrow even if the hair root has been removed right up to the level of the transient portion of the follicle.

(b) New growth of fetal eyebrow hair (4 month fetus). Sebaceous glands are already being formed during the middle of hair germ stage. Once the sebaceous gland is fully formed, the young hair starts to grow from the secretory portion of the sebaceous gland (Figs 6a, 6b, 7, 8).

DISCUSSION

Previously the following methods have been in use for obtaining thick section specimens.

(1) *Serial thin sectioned specimen method*

Paraffin embedded specimens are usually cut to around 3–5 μm thick, because with thicker sections the tissue tends to curl, fissure and split. Moreover, in a thin sectioned specimen, movement of the tissue may occur, and it is sometimes impossible to study the whole specimen without distortion.

(2) *Celloidin embedded method (Leach, 1952)*

The tissue is infiltrated with celloidin at room temperature. With this method, the contraction and hardening, which are drawbacks of paraffin embedding, do not occur. It also has the merit that specimens 200–500 μm thick can be obtained. But celloidin, being a long molecule, does not permeate the tissue easily, and therefore embedding takes a long time (about 1 month or longer). As the tissue is usually dyed without removing the celloidin, the range of staining techniques is limited. In addition, securing an unwrinkled specimen is a time-consuming process.

(3) *Freezing method (Sano, 1970)*

(a) *Thick sections from a frozen specimen.* Thick sections can be obtained from a specimen frozen by compressed CO_2 gas. A suitable thickness is 15–20 μm (sometimes 30–35 μm). This method can be used when histochemical studies are required, as the fat is not dissolved out by alcohol or other organic solvent. But since there are no supporting substances such as paraffin or celloidin, the tissue is readily broken up rendering staining and mounting difficult.

(b) *Cryostat method.* It is possible to obtain serial tissue specimens with an average thickness of 40 μm by using a cryostat.

From the above it can be seen that these methods of obtaining thick sections of tissue have their advantages and drawbacks.

To date the cellophane tape method has been used only in autoradiography for obtaining frozen sections.

Advantages of the cellophane tape method are as follows:

(a) Thick tissue specimens can be easily obtained, but, more important, it is compatible with the standard practice of embedding in paraffin. The desired thickness can be easily and surely obtained without either moving or breaking down the tissue.

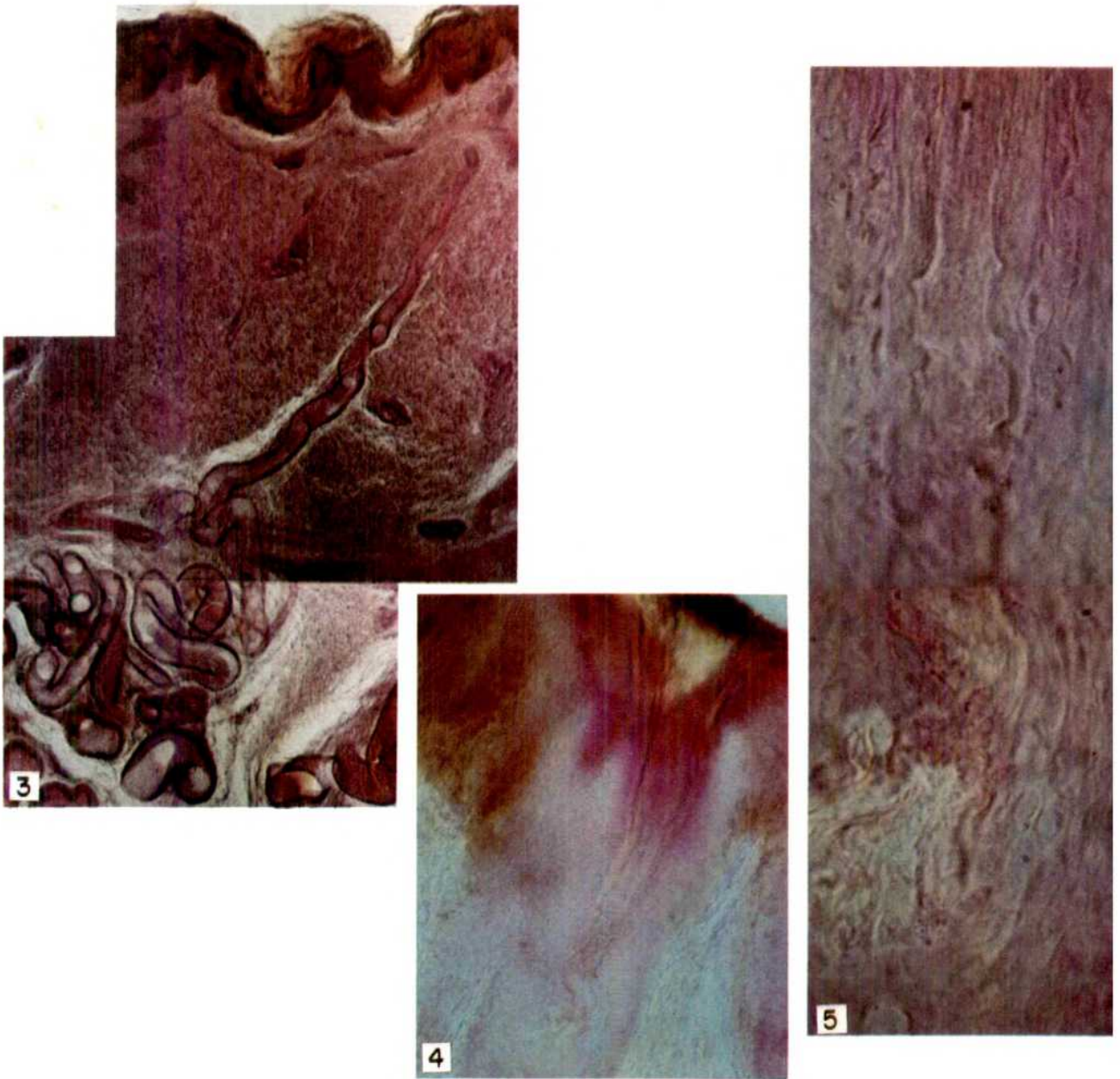


FIGURE 3. Apocrine gland on its own—the axillary hair is absent after electrocoagulation. This may indicate that loss of the sebaceous gland results in failure of the hair to regrow.

FIGURE 4. Six months after subcutaneous shaving of axilla. New young hair can be seen in the vicinity of the secretory duct of the sebaceous gland.

FIGURE 5. Enlargement of Fig. 4 (secretory portion of sebaceous gland). Mitotic activity can be seen around secretory portion of sebaceous gland. Gradual keratinization occurs and cuticle-like projections can be seen.

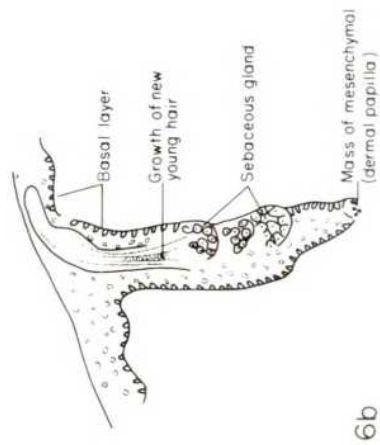
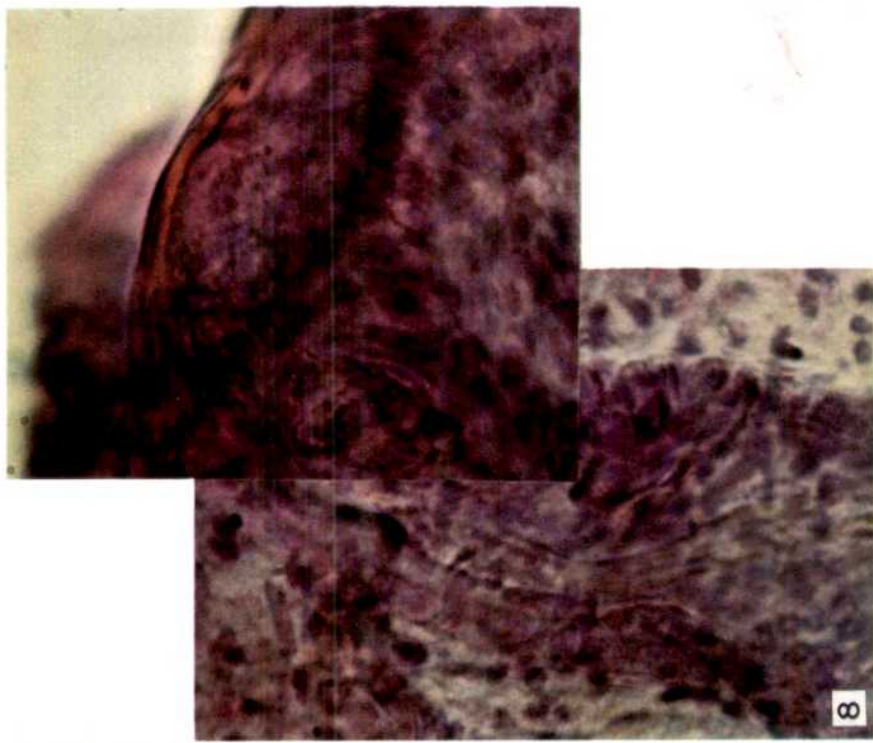
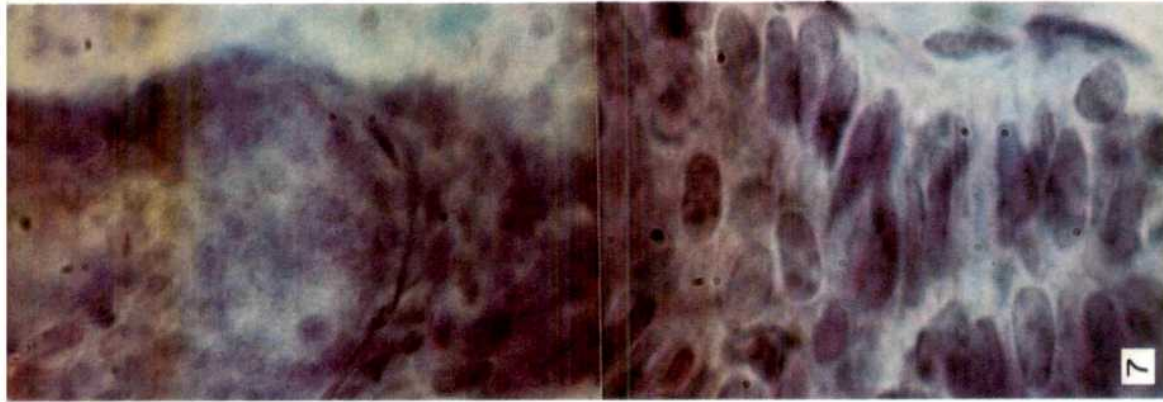


FIGURE 6. (a) Late hair germ stage in the eyebrow of 4-month-old fetus. (b) Late hair germ stage.
 FIGURE 7. Sebaceous gland formation is complete, but the hair bulb has not yet formed. (Enlargement of the lower part of the new young hair follicle in Fig. 6.)
 FIGURE 8. Formation of new young hair can be seen around secretory portion of sebaceous gland. (Enlargement of the upper part of the new young hair follicle in Fig. 6.)

- (b) Removal of the cellophane tape and paraffin is easy.
- (c) The staining procedure is in general similar to other methods but the specimen can be stained in greater detail.

CONCLUSION

- (1) Serial thick sliced tissue specimens can be easily obtained by attaching cellophane tape to the paraffin block.
- (2) Improvements in this method have facilitated completer removal of both the cellophane tape and the paraffin.
- (3) Stereo-histomorphology of the tissue can be easily studied since tissue movement does not occur and staining is good.

REFERENCES

- INABA, M. & YAMAMURA, T. (1975) Preparation of thick section specimens utilizing cellophane tape. *Clinical Dermatology, Japan*, **17**, 461.
- INABA, M. (1976) *Therapy of Hyperhydrosis and Osmidrosis*, pp. 87-88. Kinensha, Japan.
- INABA, M. (1977) New instrument for hircismus and hyperhydrosis operation. *Plastic and Reconstructive Surgery*, **59**, 864-866.
- LEACH, E.H. (1952) The staining of thick sections of skin. *British Journal of Dermatology*, **64**, 183.
- SANO, Y. (1970) *Histological Study Method*, pp. 135-141. Nanzando, Tokyo.

Pemphigoid and pemphigus antigens in cultured epidermal cells

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SUMMARY

Fractions of IgG from sera of patients with pemphigoid and pemphigus added to monolayer cultures of mouse epidermal cells resulted in a sparse distribution of cells. Direct immunofluorescence studies of these monolayers revealed epidermal cell surface antigens reacting with pemphigoid and pemphigus antibodies. We suggest that these antibodies may bind to epidermal cell surface antigenic molecules which are important in maintaining epidermal cell adhesion in culture.

Pemphigoid and pemphigus are diseases in which epidermal cell adhesion is abnormal (cell-to-cell in pemphigus and cell-to-basement membrane in pemphigoid). This defective adhesion process is associated with an auto-immune antigen/antibody reaction occurring on surfaces of epidermal cells, i.e. basal cells in pemphigoid, differentiating keratinocytes in pemphigus. We are studying the *in vitro* effects of pemphigoid, pemphigus and normal IgG fractions on mouse epidermal cells grown in culture to determine whether the epidermal cell adhesion defect and the autoimmune reaction are pathogenetically related. The present investigation was undertaken, first to determine the presence of pemphigus and pemphigoid antigens on mouse epidermal cells grown in culture, and second to detect changes in monolayer morphology that may occur as a result of the antigen/antibody reaction.

MATERIALS AND METHODS

Primary epidermal cell culture. Primary cultures of neonatal mouse epidermal cells (BALB/c) were prepared as described by Marcelo *et al.* (1977). Briefly, neonatal mouse epidermis was separated from the dermis by incubation in 0.25% trypsin (Difco, 1:250, Detroit, Michigan) for 60 min at 37°C. Gentle shaking of the isolated epidermis yielded a keratinocyte suspension that was further purified from dermis and fibroblasts by discontinuous Ficoll gradient centrifugation. The keratino-

Reprint requests to Dr L.A. Diaz.

Abbreviations: M-199, medium 199; FCS, fetal calf serum; IF, immunofluorescence; CF-II, Cohn fraction II; PBS, phosphate buffered saline.

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cytes were washed with medium 199 (M-199) + 13% fetal calf serum (FCS) + 50 IU/ml of penicillin and 50 mcg/ml of streptomycin (Flow Labs, Rockville, Maryland) and counted. 1.8×10^6 trypan blue excluding cells were inoculated into Costar plastic Leighton tubes containing a non-fluorescent plastic coverslip and M-199 + FCS to give a final volume of 2 ml. The tubes were gassed with a 5% CO₂ in air mixture and incubated at 32–33°C. Nutrients were added to the cultures every other day.

Immunofluorescent (IF) techniques. Indirect and direct IF staining was performed by previously established methods (Beutner, Chorlitzski & Jordon, 1970). Cryostat cut sections of neonatal or adult mouse tongue were used as substrates for indirect IF studies. Treated and control epidermal monolayers were also used for direct IF. Fluorescein-conjugated goat anti-human IgG serum (Molar F/P ratio 2.7, Meloy Laboratories, Springfield, Va.) was employed in both procedures. Blocking experiments were performed by pre-incubating the epidermal monolayers with unlabelled goat anti-human IgG serum (Hyland Div., Travenol Labs., Costa Mesa, Ca.) and then performing the direct IF procedure.

Sera. Pemphigus vulgaris and bullous pemphigoid sera were heat inactivated and then absorbed with human erythrocytes (A,B,O,LeA⁺, LeB⁺). The IgG fraction of each serum was purified by ammonium sulphate precipitation and DEAE-cellulose chromatography (Fahey & Terry, 1973). The purified IgG fractions were adjusted to a known protein concentration and the indirect IF titre determined (pemphigus IgG, 19.3 mg/ml, IF titre 1:320; pemphigoid IgG, 7.69 mg/ml, IF titre 1:160).

Experimental design. Four triplicate sets of cultures in Leighton tubes were prepared. Human IgG fractions were diluted in culture medium (M-199 + 13% FCS) and added to the tubes at day 0 (time of inoculation) and at each subsequent medium change. The tubes were coded as follows: (1) 300 µg/ml of human IgG (CF-II, Sigma, St. Louis, Mo.); (2) 400 µg/ml of pemphigoid IgG; (3) 500 µg/ml of pemphigus IgG; and (4) no additives. Each group was studied on days 0, 2, 4 and 7. The coverslips coated with cultured epidermal cells were cut in half; one half was used as a substrate for direct IF examination and the other was fixed with acetic acid/ethanol and stained with haematoxylin/eosin for studies of monolayer morphology.

RESULTS

Tissue substrate. Purified pemphigus and pemphigoid IgG reacted with mouse tongue epithelia producing the typical staining patterns described in other mammalian tissues (Beutner, Chorlitzski & Jordon, 1970).

Epidermal cell suspension. On day 0, the suspension of epidermal cells displayed some variation in the degree of binding with pemphigus and pemphigoid antibodies. Some cells showed the staining patterns previously described by others (Brigden & Amos, 1975). However, in other batches of cells, these staining features were absent or difficult to differentiate from those produced by normal human IgG (CF-II).

Epidermal monolayer morphology. The normal growth and differentiation of neonatal mouse epidermal cells grown in culture have been described in detail by Marcelo *et al.* (1977), employing light microscopy, scanning and transmission electron microscopy as well as histochemical techniques. Briefly, under ideal conditions, the epidermal cells will cover about 80% of the surface substrate by day 2. Individual cells are hexagonal with a large nucleus and a rough cell surface. By day 3 or 4 a complete monolayer is formed as seen in Fig. 1a. Culture stratification is seen by day 5 or 6, and by day 12–14 there is a massive 'pile up' of 'rounded bodies' which are degenerating specialized cells.

Control monolayers. Epidermal cells grown in medium containing human IgG (CF-II) or in IgG-free medium showed normal monolayer morphology throughout the study (Figs 1a, 1b, 2a, 2b).

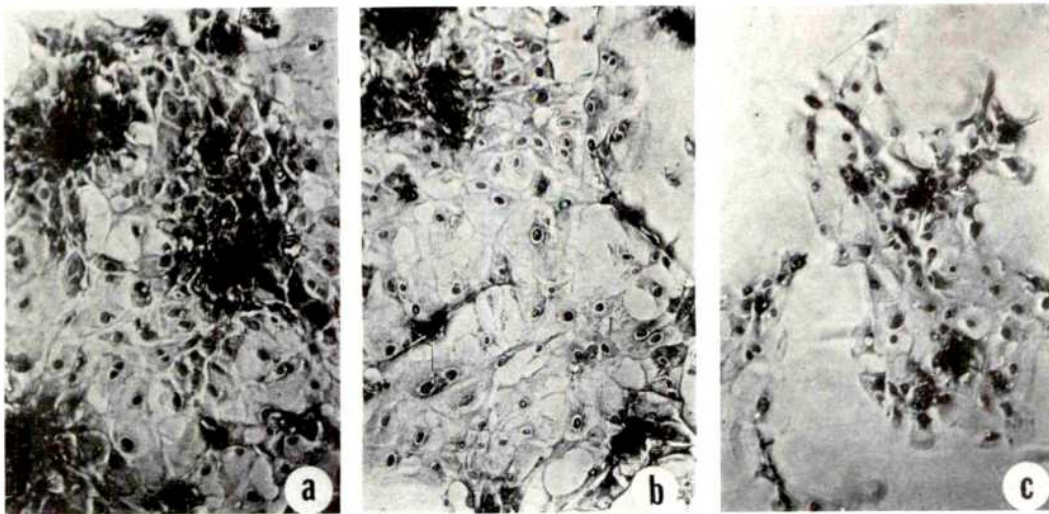


FIGURE 1. Epidermal monolayers (4 days after inoculation); (a) grown in human IgG-free media, (b) grown in human IgG (CF-II), and (c) grown in pemphigoid IgG ($\times 115$).

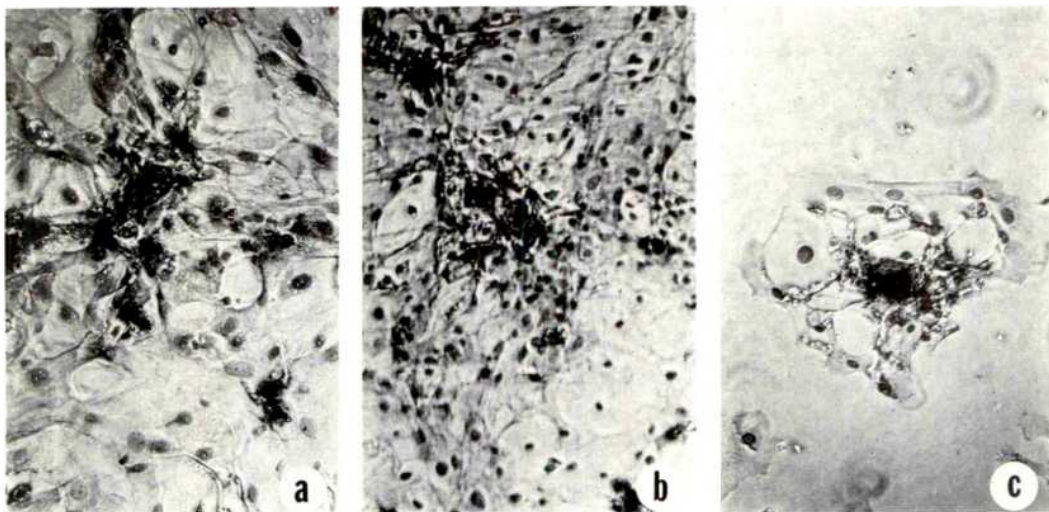


FIGURE 2. Epidermal monolayers (7 days after inoculation); (a) grown in human IgG-free media ($\times 230$), (b) grown in human IgG (CF-II) ($\times 115$), and (c) grown in pemphigus IgG ($\times 230$).

Direct IF studies of these monolayers revealed a yellowish-brown staining of the cells on days 2, 4, and 7 that was interpreted as a negative result (Fig. 3a). Some degenerated epidermal cells attached to the surface of the monolayer stained diffusely.

Pemphigoid-treated monolayers. Most monolayers treated with pemphigoid IgG showed a marked sparseness on days 2 and 4. Less than 60% of the substrate surface was covered by cells (Fig. 1c). These cells were seen in colonies or as single elements. However, several pemphigoid IgG-treated cultures, after this initial change, resembled the cellular density of control cultures by day 7. Through-

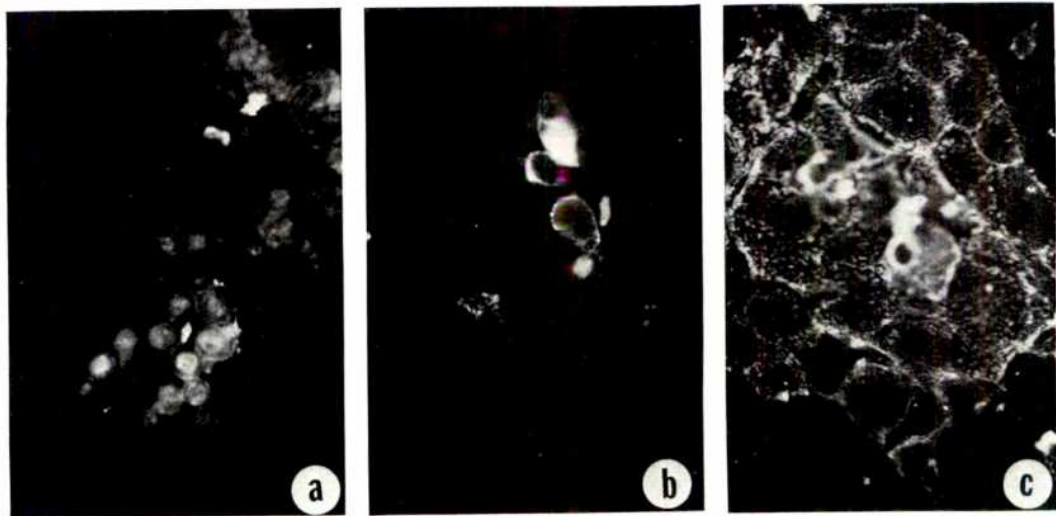


FIGURE 3. Direct IF studies of epidermal monolayers grown in human IgG fractions (2 days after inoculation); (a) IgG (CF-II), (b) pemphigoid IgG, and (c) pemphigus IgG ($\times 310$).

out the experiment, the cells which did attach to the coverslip showed normal morphology. By direct IF examination only a small number of the epidermal cells comprising the monolayers fixed pemphigoid IgG. These single cells were usually found in the space separating colonies of epidermal cells. The reacting cells were oval or elongated showing a granular staining of the cell surface. Occasionally these cells showed a polar rim of fluorescence (Figs 3b & 4a). Few cells bound pemphigoid antibodies, approximately 3 per field ($\times 310$) on day 2. Such cells were scarce on day 4 and only

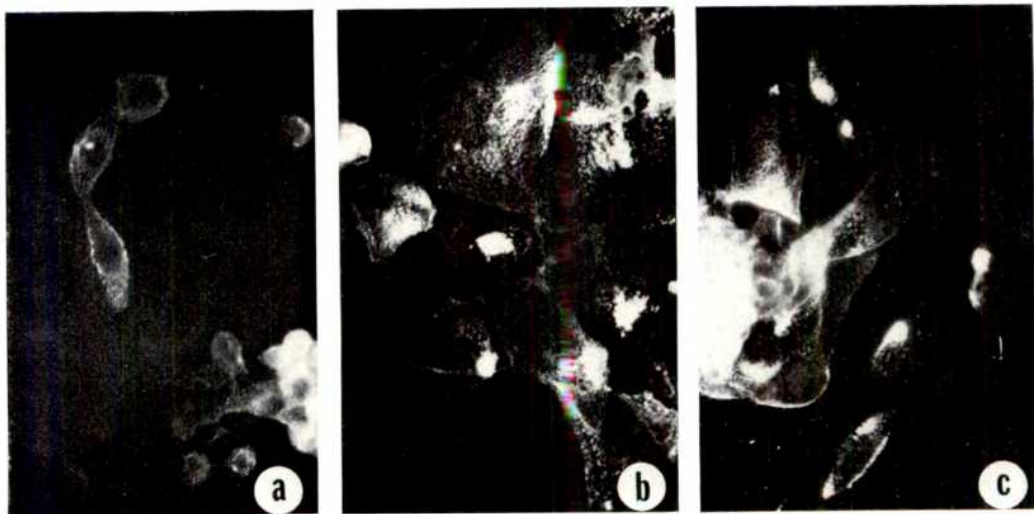


FIGURE 4. Direct IF studies of epidermal monolayers grown in human IgG fractions (4 days after inoculation); (a) pemphigoid IgG, (b and c) pemphigus IgG ($\times 310$).

occasionally found on day 7. The staining of the epidermal cells was specifically abolished by pre-treatment of the monolayer with unlabelled goat antihuman IgG. Colonies of epidermal cells scattered on the slide showed no fluorescence.

Pemphigus-treated monolayers. Monolayers treated with pemphigus antibodies showed normal development until day 4, when some of the cultures became sparse. By day 7, most cultures showed altered morphology and were sparse (Fig. 2c). The attached epidermal cells in these sparse monolayers were flat and scattered on the coverslip surface. No evidence of stratification was observed. The surface of plated epidermal cells reacted by direct IF with pemphigus antibodies producing staining patterns that changed as the culture grew older. On day 2, keratinocytes grouped in colonies reacted with pemphigus antibodies giving a honeycomb appearance to the cell colony (Fig. 3c). The surface staining of the individual cells was granular and irregular (Fig. 3c). On days 4 and 7, the surface of the cells which remained attached to the substrate appeared granular and clumpy. The reacting antigens in these cells coalesced into an amorphous mass as seen in Figs 4b and 4c. The staining of the cell surface produced by pemphigus antibodies was prevented by pre-incubation of the monolayers with unlabelled goat antihuman IgG sera.

DISCUSSION

This study has shown, firstly, that the addition of pemphigoid and pemphigus IgG fractions alter the monolayer morphology of neonatal mouse epidermal cells grown in culture. Untreated control cultures or cultures treated with normal human IgG (CF-II) did not show these changes. The addition of pemphigoid and pemphigus IgG fractions caused the monolayers to become sparse. The changes produced by the pemphigoid IgG fraction were detected mainly on days 2 and 4, whereas pemphigus IgG treated cultures showed alterations in morphology beginning on day 4. A direct complement-dependent cytotoxic effect of these autoantibodies on epidermal cells may account for the low density of cells coating the coverslip substrate. However, another attractive explanation for this phenomenon is that pemphigoid and pemphigus antibodies bind cell surface molecules which are important in maintaining epidermal cell adhesion in culture. Both possibilities are being investigated. Pemphigus antibodies have been found to be noncytotoxic to guinea-pig epidermal cells (Takigawa & Imamura, 1977). However, pemphigus IgG fractions are known to induce acantholysis in epidermis grown in the organ culture system (Schlitz & Michel, 1976).

The second finding reported in this paper is the detection by direct IF of antigens reacting with pemphigoid and pemphigus antibodies on the surface of epidermal cells grown in culture. The specificity of this reaction was verified by blocking experiments with unlabelled goat antihuman IgG. Neither untreated epidermal cells nor cells treated with normal human IgG (CF-II) showed surface fluorescence. Pemphigus and pemphigoid antigens detected on epidermal cells in suspension (day 0) showed IF patterns previously reported (Brigden & Amos, 1975). However, we found that these features on occasion were absent or difficult to differentiate from those produced by normal human (CF-II). These variable results may depend on the amount of antigen available on the epidermal cell surface on day 0. Trypsinization and repeated washing during experimental procedures may remove these antigens which are known to be PBS-soluble (Shu & Beutner, 1973; Diaz *et al.*, 1977). Several presently unknown factors may account for the detection of a small and decreasing number of epidermal cells bearing pemphigoid antigen, such as the limits of sensitivity of IF techniques, the decreasing number of cells producing this antigen as a culture grows older, and that most cells after binding pemphigoid antibodies are unable to plate normally for the reasons previously mentioned and therefore slough off the substrate during culture and IF procedures.

Pemphigus antigen, however, was present on the surface of cells grouped in colonies, producing a

honeycomb appearance. Starting at day 4, the granular pemphigus antigen agglutinated on the cell surface producing an amorphous mass. This phenomenon probably represents a capping-like effect described in other cell surface antigen/antibody systems (Carey, Kuhn & Hardford, 1965; Yefenoy & Klein, 1974; Schreiner & Unanue, 1976). It is unclear if this phenomenon is related to the onset of cellular detachment observed in pemphigus treated monolayers.

Several investigations confirm the importance of certain cell surface antigens in the adhesion or aggregation phenomenon of cells (Moscona, 1974; Pearlstein, 1976; Rutishauser *et al.*, 1976; Yamada, Yamada & Pastan, 1976; Klebe *et al.*, 1977; Poussegur, Willingham & Pastan, 1977; Öbrink, Kuhlenschmidt & Roseman, 1977; Ali *et al.*, 1977). Furthermore, antibodies against these cell surface antigens when added to cells bearing such antigens are known to impair the cell adhesion or aggregation of these cells (Ali *et al.*, 1977; O'Neill & Follett, 1970; Beug, Katz & Gerisch, 1973; Rosen, Haywood & Barondes, 1976), to alter phagocytosis (Carey & Pettengill, 1967) and to induce morphological changes in these cells causing them to become rounded (Yamada *et al.*, 1976; Ali *et al.*, 1977; O'Neill & Follett, 1970). It is important to note that acantholytic cells found in pemphigus lesions are rounded and demonstrate surface bound antibodies (Wolff & Schreiner, 1971). A hypothesis being explored experimentally in our laboratory is that pemphigoid and pemphigus antigens are important in maintaining epidermal cell-to-cell (pemphigus) and cell-to-basement membrane (pemphigoid) adhesion. The antibodies produced by afflicted patients would interfere with epidermal cell adhesion *in vivo*.

ACKNOWLEDGMENT

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REFERENCES

- ALI, I.U., MAUTNER, V., LANZA, R. & HYNES, R.O. (1977) Restoration of normal morphology, adhesion and cytoskeleton in transformed cells by addition of a transformation-sensitive surface protein. *Cell*, **11**, 115.
- BEUTNER, E.R., CHORZEKSKI, T.P. & JORDON, R.E. (1970) *Autosensitization in Pemphigus and Bullous Pemphigoid*. Charles C. Thomas, Springfield, Ill.
- BEUG, H., KATZ, F.E. & GERISCH, G. (1973) Dynamics of antigenic membrane sites relating to cell aggregation in *Dictyostelium discoideum*. *Journal of Cell Biology*, **56**, 647.
- BRIGDEN, W.D. & AMOS, H.E. (1975) The differential binding of antibody from the sera of patients with pemphigus and pemphigoid to isolated guinea-pig epidermal cells. *British Journal of Dermatology*, **93**, 425.
- CAREY, F.J., KUHN, N.O. & HARFORD, C.G. (1965) Effects of anticellular serum on phagocytosis and the uptake of tritiated thymidine and uridine by HeLa cells. *Journal of Experimental Medicine*, **121**, 991.
- CAREY, F.J. & PETTINGILL, O.S. (1967) Time-lapse study of effects of antibody in membrane mobility and phagocytic activity of HeLa cells. *Journal of Cell Biology*, **33**, 709.
- DIAZ, L.A., CALVANICO, N.J., TOMASI, T.B., Jr & JORDON, R.E. (1977) Bullous pemphigoid antigen: isolation from normal human skin. *Journal of Immunology*, **118**, 455.
- FAHEY, J.L. & TERRY, E.W. (1973) Ion exchange chromatography and gel filtration. In: *Handbook of Experimental Immunology, Immunochemistry* (Ed. by D.M. Weir), Vol. 1, p. 7-1 Blackwell Scientific Publications, Oxford.
- KLEBE, R.J., ROSENBERGER, P.G., NAYLOR, S.L., BURNS, R.L., NOVAK, R. & KLEINMAN, H. (1977) Cell attachment to collagen. Isolation of a cell attachment mutant. *Experimental Cell Research*, **104**, 119.
- MARCELO, C.L., KIM, Y.G., KAINE, J. & VOORHEES, J.J. (1977) Stratification and specialization of primary keratinocyte culture: Evidence of a functioning *in vitro* epidermal cell system. *Submitted for publication*.
- MOSCONA, A.A. (1974) Surface specification of embryonic cells: lectin receptors cell recognition and specific cell ligands. In: *The Cell Surface Development* (Ed. by A.A. Moscona), p. 67. John Wiley and Sons, New York.
- O'NEILL, C.H. & FOLLETT, E.A.C. (1970) An inverse relation between cell density and the number of microvilli in cultures of BHL 21 hamster fibroblasts. *Journal of Cell Science*, **7**, 685.

- ÖBRINK, B., KUHLENSCHMIDT, M.S. & ROSEMAN, S. (1977) Adhesive specificity of juvenile rat and chicken liver cells and membranes. *Proceedings of the National Academy of Sciences, U.S.A.*, **74**, 1077.
- PEARLSTEIN, E. (1976) Plasma membrane glycoprotein which mediates adhesion of fibroblasts to collagen. *Nature*, **262**, 497.
- POUYSSÉGUR, J., WILLINGHAM, M. & PASTAN, I. (1977) Role of cell surface carbohydrates and proteins in cell behavior: Studies on the biochemical reversion of an N-acetylglucosamine-deficient fibroblast mutant. *Proceedings of the National Academy of Sciences, U.S.A.*, **74**, 243.
- RUTISHAUSER, U., THIERY, J.P., BRACKENBURY, R., SELA, B.A. & EDELMAN, G.D. (1976) Mechanisms of adhesion among cells from neural tissues of chick embryo. *Proceedings of the National Academy of Sciences, U.S.A.*, **73**, 577.
- ROSEN, S.D., HAYWOOD, P.L. & BARONDES, S.H. (1976) Inhibition on intercellular adhesion in a cellular slime mold by univalent antibody against a cell-surface lectin. *Nature*, **263**, 425.
- SHU, S. & BEUTNER, E.H. (1973) Isolation and characterization of antigens reactive with pemphigus antibodies. *Journal of Investigative Dermatology*, **61**, 270.
- SCHLITZ, J.R. & MICHEL, B. (1976) Production of epidermal acantholysis in normal human skin *in vitro* by the IgG fraction from pemphigus serum. *Journal of Investigative Dermatology*, **67**, 254.
- SCHREINER, G.F. & UNANUE, E.R. (1976) Membrane and cytoplasmic changes in B lymphocytes induced by ligand-surface immunoglobulin interaction. *Advances in Immunology*, **24**, 38.
- TAKIGAWA, M. & IMAMURA, S. (1977) Experimental production of rabbit anti-guinea-pig epidermal cell sera. Comparison to pemphigus antibodies. *Journal of Investigative Dermatology*, **68**, 254.
- WOLFF, K. & SCHREINER, E. (1971) Ultrastructural localization of pemphigus auto-antibodies within the epidermis. *Nature*, **229**, 59.
- YEFENOF, E. & KLEIN, G. (1974) Antibody induced redistribution of normal and tumor associated surface antigens. *Experimental Cell Research*, **88**, 217.
- YAMADA, K.M., YAMADA, S.S. & PASTAN, I. (1976) Cell surface protein partially restores morphology, adhesiveness, and contact inhibition of movement to transformed fibroblasts. *Proceedings of the National Academy of Sciences, U.S.A.*, **74**, 1077.

Quantitative histological changes during early experimental carcinogenesis in the hamster cheek pouch

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SUMMARY

Sequential changes in the hamster cheek pouch epithelium following application of the carcinogen dimethyl benzantracene have been assessed using stereological techniques. The most important progressive change found following cessation of carcinogen painting appears to be an increase in the number and size of progenitor cells. Much of the marked increase in stratum corneum thickness seen in the early stages appeared to be related to a hyperplasiogenic rather than a neoplasigenic action of the carcinogen.

The hamster cheek pouch system is reported to be a reliable model of oral carcinogenesis (Salley, 1954; Morris, 1961). Many workers have subjectively described the histopathological changes in the epithelium following carcinogen applications (see Shklar, 1972 and Homburger, 1972 for reviews) but there is a paucity of quantitative information, especially during the preneoplastic stages. Assessment of such changes may prove to be of value in extending knowledge of premalignant lesions.

The aim of the present investigation was to quantify early sequential changes in hamster cheek pouch epithelium following carcinogen applications. The parameters examined were changes in total epithelial thickness and in the thickness of epithelial compartments, and changes in cell numbers and cell sizes within individual epithelial compartments.

MATERIALS AND METHODS

Twenty line-bred, male Syrian golden hamsters (*Mesocricetus auratus*)* aged between 8 and 10 weeks were used in the experiment. The carcinogen was a 0.25% solution of 9,10-dimethyl-1,2-benzanthracene (DMBA) in acetone. This concentration of DMBA was half that used in most previous investigations of carcinogenesis in the cheek pouch. The weaker concentration was selected as it increases the latent period (Morris, 1961), thus extending the time during which preneoplastic

* Supplied by Coombehurst Breeding Establishment, Basingstoke, Hampshire.

changes are likely to be seen, but does not significantly decrease the tumour yield (MacDonald, 1978) relative to the more generally used concentration. In addition, the weaker concentration decreases the likelihood of ulceration with the concomitant problems in histological interpretation due to scarring.

Animals were lightly anaesthetized with ether and held in a restrainer of the type described by Moss, Collins & Cole (1965). The carcinogen was applied to the medial wall of the pouch by a single stroke of a 1 cm wide brush using a wiped-brush technique (Morris, 1961). A short blast of air was used to evaporate the acetone solvent leaving a crystalline residue of carcinogen adherent to the desired area of mucosa. Pouches were painted three times weekly for a maximum of 6 weeks.

Five animals were untreated controls and the remainder were divided into three groups, each of five animals. The first group was killed after 3 weeks of painting and the second group after 6 weeks of painting. The remaining animals were killed 3 weeks after the last carcinogen application.

At the time of killing an incisional biopsy approximately 10×3 mm was taken from the treated area of mucosa. This was cut at right angles to the epithelial surface into 3×1 mm blocks. As the material used for quantitative analysis was also to be used in a related cell-kinetic study, blocks were incubated with tritiated thymidine for double labelling using the technique described by Warnakulasuriya, MacDonald & Rennie (1975). Blocks were fixed in Bouin's fluid for 2 h and then transferred to 70% methylated spirit. Randomly selected blocks were paraffin processed. Sections were cut at $3.5 \mu\text{m}$ and stained with haematoxylin and eosin. Random sections were used for stereological analysis (Weibel, 1969).

It was felt possible that the techniques of biopsy and specimen preparation might result in loss of superficial squames from the stratum corneum. Care was taken at the biopsy stage to avoid touching the keratinized surface of the biopsy site. At all stages subsequently the material was handled with minimal agitation. When the histological sections were examined, in many instances red blood cells were present on the keratinized surface strongly suggesting that loss of surface squames had not occurred. Accordingly, no special precautions, other than those noted above, were taken to avoid such loss.

The pouch mucosa of the hamster is divisible into progenitor, maturation and keratinized compartments (MacDonald, 1971). Distinction between the maturation and keratinized compartments was readily made, but distinction between the maturation and progenitor compartments was less easy. The distinction was made subjectively by consideration of features of cell size, nucleo-cytoplasmic ratio, orientation and staining characteristics. Progenitor cells were smaller and darker staining with a smaller volume of cytoplasm giving a greater nucleo-cytoplasmic ratio than maturation compartment cells. The nuclei of maturation cells were larger and showed paler, vesicular staining. Progenitor cells tended to be elongated at right angles to the basement membrane, often with a palisaded arrangement, while maturation cells were flattened with the long axis at right angles to the progenitor cells and parallel to the surface.

In normal pouch mucosa, the progenitor compartment is restricted to a single basal layer of cells. In carcinogen treated mucosa, most of the cells fulfilling the suggested criteria for progenitor cells were in the deepest three cell layers and the presence of mitoses and of labelled cells in autoradiographs confirmed this distribution.

An image of the section was projected on to the screen of a Leitz teaching head and lines of demarcation were drawn between the epithelial compartments on the screen. A lattice grid of 5 mm squares was used to make stereological point counts. The grid was rotated to three positions to overcome problems of anisotropy (Weibel, 1969) and to give sufficient numbers of points for the results to fall within 10% of the accumulative means (Schroeder & Munzel-Pedrazzoli, 1970). Columns of epithelium of known width (0.19 mm) and running through the full epithelial thickness at right angles to the epithelial surface were counted. By noting the number of points falling on each compart-

ment, it was possible to calculate the area of each anc , by dividing these areas by the column width, a mean thickness for the total epithelium or individual compartments was obtained.

Cell numbers were estimated by counting the total numbers of viable nuclei in each compartment and applying Abercrombie's correction (Abercrombie, 1946). By using the corrected cell numbers and the area measurements obtained from point counting it was possible to estimate mean cell section areas. This measurement was a slight overestimate of cell size as the intercellular spaces were included in the measurements.

Comparisons between groups of animals were made using non-parametric statistical methods because the small numbers in individual groups made it impossible to satisfy the assumptions required

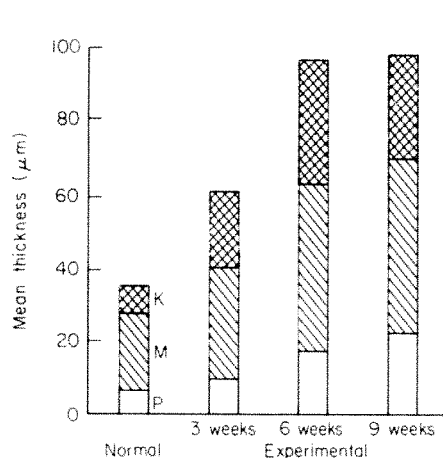


Fig. 1.

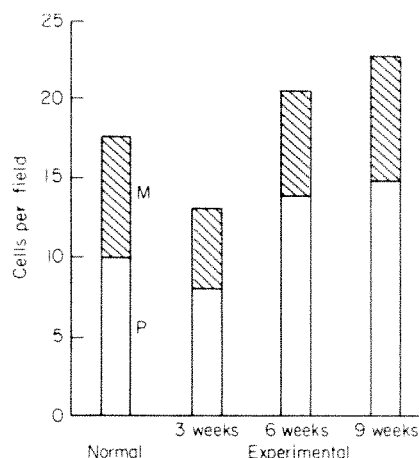


Fig. 2.

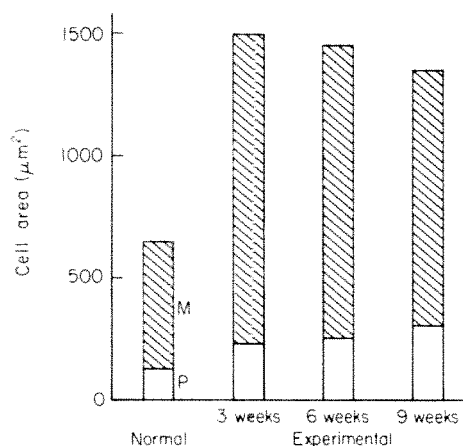


Fig. 3.

FIGURE 1. Mean epithelial thickness following application of DMBA. The proportion of the total thickness formed by each compartment is indicated by P, progenitor; M, maturation; and K, keratinized compartments.

FIGURE 2. Corrected cell numbers in progenitor and maturation compartments. Each field is a column of 0.19 mm width running through the thickness of epithelium at right angles to the epithelial surface.

FIGURE 3. Cell size expressed as mean cell section area for cells in progenitor and maturation compartments.

for parametric methods. All probabilities cited in the results have been derived from Mann-Whitney U tests.

RESULTS

Fig. 1 shows the changes in total mean epithelial thickness. After 3 weeks of carcinogen application there was a nearly twofold increase in mean epithelial thickness ($P=0.004$). By 6 weeks there was a further significant increase in epithelial thickness ($P=0.004$). At 9 weeks, that is 3 weeks after the last carcinogen application, there had been no further significant change in epithelial thickness. Fig. 1 also shows changes in the mean thickness of individual epithelial compartments. The increase in thickness at 3 weeks was due mainly to an increase in the size of the keratinized and maturation compartments ($P=0.008$) with a relatively minor, but still significant, increase in the size of the progenitor compartment ($P=0.016$). By 6 weeks the progenitor compartment was twice the normal thickness ($P=0.004$) and there were further increases in the maturation compartment ($P=0.008$) and keratinized compartment ($P=0.004$). Although the total thickness of epithelium was unchanged at 9 weeks there was a reduction in the thickness of the keratinized compartment and a corresponding increase in the thickness of the cellular compartments, mainly the progenitor cell compartment ($P=0.008$).

Fig. 2 shows the corrected cell numbers in the progenitor and maturation compartments. At 3 weeks there was a fall in the number of cells in both compartments, especially the maturation cells ($P=0.004$). By 6 weeks the number of cells in the maturation compartment had returned to control levels, but the number of progenitor cells was significantly above control levels ($P=0.004$). There was no further change at 9 weeks.

Fig. 3 shows changes in cell sizes. At each of the three experimental periods there was a marked increase in the size of progenitor cells as compared with controls ($P=0.004$) and this change appeared to be progressive. The size of cells in the maturation compartment was also significantly above control levels ($P=0.004$) from 3 weeks onwards. The apparent fall in cell size after 3 weeks was not significant due to wide variation between individual animals.

DISCUSSION

Some of the quantitative changes found in this investigation are shown in the series of illustrative biopsies in Fig. 4, all of which are at the same magnification.

At 3 weeks the epithelium was in the inflammatory phase (Salley, 1957) and was twice the normal thickness. The stratum corneum was three times the normal thickness. The increase in the size of the cellular compartments was due to increases in the size of individual progenitor and maturation cells, their numbers in fact being reduced. Subjectively it was felt that there was evidence of cell death and degeneration within the epithelium.

At 6 weeks the epithelium was further increased in thickness, but the inflammatory reaction was less severe. There was a considerable increase in stratum corneum thickness. The increase in the thickness of the cellular compartments was due to a recovery in the numbers of cells in the maturation compartment and an increase in the numbers, and to a lesser extent size, of the progenitor cells.

At 9 weeks, 3 weeks after the last carcinogen application, there had been no change in the overall epithelial thickness, but there was a decrease in the size of the keratinized compartment and a corresponding increase in the size of the cellular compartments. This suggests that much of the early increase in stratum corneum thickness was due to a hyperplasiogenic rather than neoplasiogenic action of the carcinogen. The increase in the thickness of the cellular layers appeared to be due to a pro-

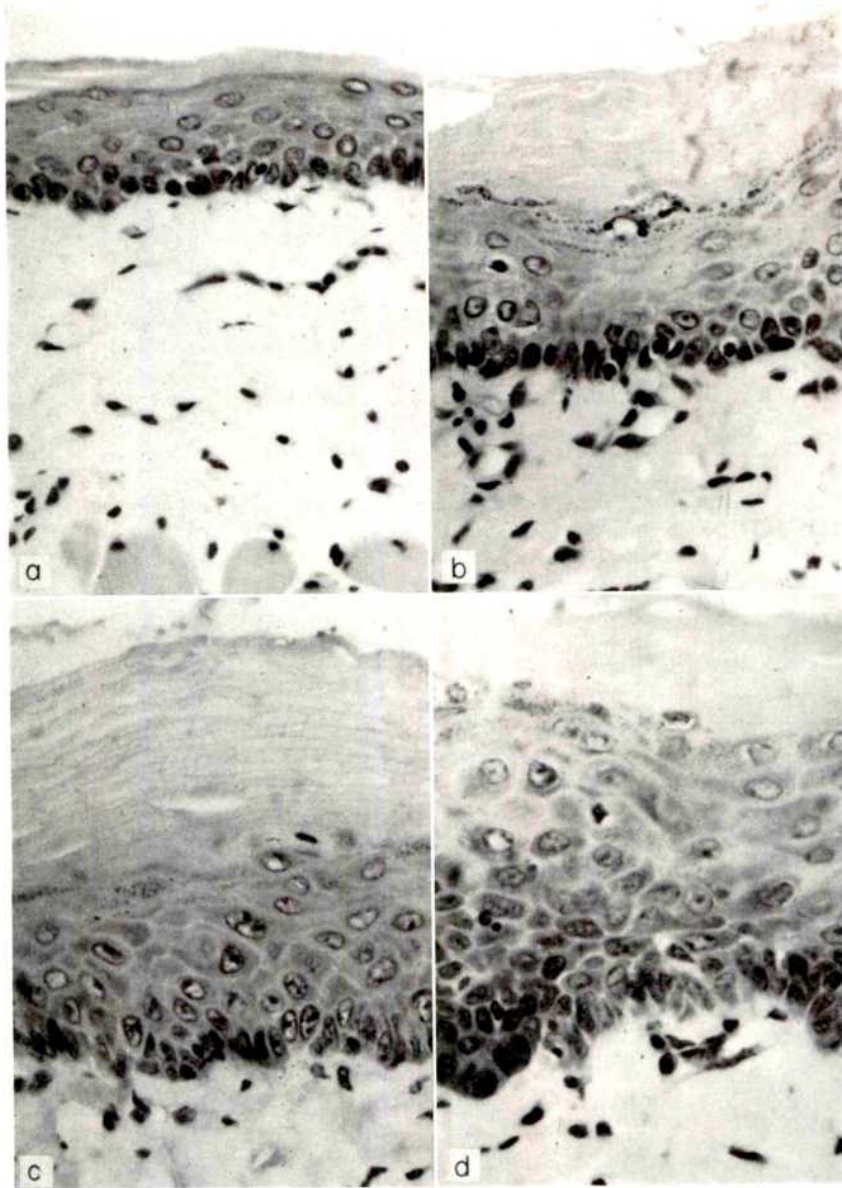


FIGURE 4. Typical areas of epithelium from normal and DMBA treated pouch. All photomicrographs are at the same magnification ($\times 400$). (a) Normal; (b) 3 weeks; (c) 6 weeks; (d) 9 weeks.

gressive increase in the size and number of progenitor cells. This is the quantitative expression of one of the suggested features of epithelial atypia in oral mucosa; namely basal cell hyperplasia (Smith & Pindborg, 1969). In the cheek pouch epithelium this might be more appropriately called progenitor cell hyperplasia and hypertrophy.

REFERENCES

- ABERCROMBIE, M. (1946) Estimation of nuclear population from microtome sections. *Anatomical Record*, **94**, 239.
- HOMBURGER, F. (1972) Chemical carcinogenesis in the Syrian hamster. In: *Progress in Experimental Tumour Research* (Ed. by F.Homburger), Vol. 16, p. 152. Karger, Basel.
- MACDONALD, D.G. (1971) Cell renewal in oral epithelium. In: *Current Concepts of the Histology of Oral Mucosa* (Ed. by C.A.Squier and J.Meyer), p. 61. Charles C. Thomas, Springfield.
- MACDONALD, D.G. (1978) A technique for localization of tumours in hamster cheek pouch carcinogenesis. *Archives of Oral Biology*, in press.
- MORRIS, A.L. (1961) Factors influencing experimental carcinogenesis in the hamster cheek pouch. *Journal of Dental Research*, **40**, 3.
- MOSS, R.L., COLLINS, T.A. & COLE, D.F. (1965) A new apparatus for intra-oral manipulation in the hamster. *Journal of Dental Research*, **44**, 668.
- SALLEY, J.J. (1954) Experimental carcinogenesis in the cheek pouch of the Syrian hamster. *Journal of Dental Research*, **33**, 253.
- SALLEY, J.J. (1957) Histological changes in the hamster cheek pouch during early hydrocarbon carcinogenesis. *Journal of Dental Research*, **36**, 48.
- SCHROEDER, H.E. & MUNZEL-PEDRAZZOLI, S. (1970) Application of stereologic methods to stratified gingival epithelium. *Journal of Microscopy*, **92**, 179.
- SHKLAR, G. (1972) Experimental oral pathology in the Syrian hamster. In: *Progress in Experimental Tumour Research* (Ed. by F.Homburger), Vol. 16, p. 518. Karger, Basel.
- SMITH, C.J. & PINDBORG, J.J. (1969) *Histological Grading of Oral Epithelial Atypia by the Use of Photographic Standards*. C. Hamburgers Bogtrykkeri, Copenhagen.
- WARNAKULASURIYA, K.A.A.S., MACDONALD, D.G. & RENNIE, J.S. (1975) Cell kinetics of normal human buccal epithelium. *Journal of Dental Research*, **54**, Special Issue A, p. L123 (Abstract).
- WEIBEL, E.R. (1969) Stereological principles for morphometry in electron microscopy. *International Review of Cytology*, **26**, 235.

Primary mucinous carcinoma of the skin

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SUMMARY

Only twenty-seven cases of primary mucinous carcinoma of the skin have been reported. Three additional cases are included in this paper. The clinical presentation is distinctive: they are found most commonly in middle-aged, black males in the head and neck region as painless nodules, usually less than 3 cm in diameter. Previous reports have indicated the probable sweat gland origin of these tumours; one of our cases demonstrates a transition from a solid hidradenoma-like pattern to nests of tumour cells floating in mucin lakes, adding support for the sweat gland hypothesis. The clinical behaviour is relatively benign; late recurrences are common but metastases are rare. Only one case had widespread metastases. Although the histological appearance is distinctive, other primary sites first must be excluded. Local excision is the treatment of choice.

Primary mucin producing carcinomas of the skin are rare; most mucinous carcinomas presenting in the skin are subsequently found to be metastatic. The largest series of primary mucinous carcinomas, reported by Mendoza & Helwig (1971), comprised 14 cases spanning 27 years; only thirteen others have been reported (See Table 1). The sweat-gland origin of these tumours has been supported by several authors using histochemical staining reactions, enzyme histochemistry, and electron microscopy. A pattern of nests and cords of tumour cells forming small lumens floating in large mucin lakes has been seen in many instances. The purpose of this paper is to include three additional cases, two with long term follow-up. One of our cases will demonstrate a transition from a solid hidradenoma-like pattern to the typical pattern noted above, adding additional support to the theory of sweat gland histogenesis. The pathological implications of such a transitional phase will be discussed. A review of the previously reported cases with a discussion of the clinical characteristics, and gross and microscopic pathological presentation, is also presented.

MATERIALS AND METHODS

The three current cases were taken from the files of the Division of Surgical Pathology, Barnes and Jewish Hospitals, St Louis, Missouri. Sections from the tumours were stained with haematoxylin

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TABLE I

Case	Age	Sex	Race	Location	Follow-up	Recurrence
Mendoza & Helwig (1971)	26	M	B	Foot	Rec.†	4 years
	59	M	J	Eyelid	2 recs.	5 years
	69	M	—	Abd. wall	No FUP*	
	76	M	W	Face	2 recs.	2 years
	64	M	B	Chin	No FUP	
	66	M	W	Axilla	Lymph node met. (local)	1 year
	72	F	W	Scalp	No FUP	
	53	M	B	Cheek	Rec.	2 years
	57	M	B	Chest	No FUP	
	8	M	B	Chest wall	No FUP	
	43	M	—	Ear	No FUP	
	69	F	W	Scalp	No FUP	
	59	M	B	Cheek	Rec. 7 yrs. FUP No rec.	8 months
Lennox, Pearse & Richards (1952)	57	M	W	Eyelid	Rec.	5 years
	67	F	W	Scalp	7 yr. FUP No rec.	
Grossman & Izuno (1974)	65	M	W	Neck	2 yr. FUP No. rec.	
Rodrigues <i>et al.</i> (1973)	52	M	B	Lower eyelid	1 yr. FUP No. rec.	
Lund (1957)	53	M	B	Axilla	No FUP	
Wolfe & Sternberg (1954)	62	F	—	Scalp	2 yr. FUP No rec.	
Miller (1967)	60	M	—	Axilla	1 rec.	
Metz <i>et al.</i> (1974)	63	M	—	Face	Met. to lymph node	6 months
Headington (1977)	44	F	—	Scalp	1 yr. FUP No rec.	
	63	M	—	Axilla	3 yr. FUP No rec.	
Berg & McDivitt (1968)	70	M	—	Scalp	19 yr. FUP several rec.	
	—	—	—	Axilla	Met. Lymph node No FUP	
	—	—	—	Face	1 Rec.	
Yeung & Stinson (1977)	59	M	W	Axilla	Widespread metastasis	
Ours	63	M	B	Chin	2 Recs.	4 years
	80	M	B	Cheek	1 yr. FUP No Rec.	
	67	M	B	Cheek	1 Rec.	4 years

*Follow-up; † Recurrence.

and eosin and examined. Formalin-fixed, paraffin embedded tissue sections from Case 2 were stained with the following methods: PAS reaction with and without diastase digestion, alcian blue method at pH 2.5 and 1.0, Mayer's mucicarmine stain, and colloidal iron reaction. The alcian blue, mucicarmine, and colloidal iron stains were all prepared with and without hyaluronidase and sialidase digestion for

1h at 37°C. An additional case of basal cell carcinoma with mucinous stroma was prepared and studied in a similar manner to compare stromal and epithelial mucin reactions. All staining methods were performed following those described in the AFIP manual of histochemical techniques (Luna, 1968). Sections stained with Mayer's mucicarmine stain were available from Cases 1 and 3.

CASE REPORTS

Case 1

A 72-year-old black male presented to Jewish Hospital in July, 1967, with complaints of nausea, vomiting, and gastric distress considered to be peptic ulcer disease. In hospital a 10×10×10 mm pigmented nodule was removed from his right chin, and was diagnosed as an incompletely excised nodular hidradenoma. He returned in June, 1971, with an ulcerated and fungating 20×15 mm tumour in the same location. A diagnosis of mucinous carcinoma with cellular areas similar to the initial biopsy was made. The patient did well until September, 1976, when he returned with a second recurrence present for at least 1 year. The tumour extended to the periosteum of the mandible and was incompletely excised. No other primary site has been demonstrated and the patient is without recurrence at time of writing.

Gross pathology. The tumour was described as a cystic bluish mass which on cut section had a dull grey-tan gelatinous appearance.

Microscopic examination. The original tumour (1967) was composed of multiple nodules separated by irregular fibrous septae (Fig. 1). The nodules were composed of uniform cuboidal to columnar cells with copious eosinophilic cytoplasm lining thin fibrovascular stalks. Neither pleomorphism nor mitoses were present. Scattered through the tumour small cystic spaces were found containing pink proteinaceous material (Fig. 2). Rare mucicarmine positive material was present. The recurrent tumours demonstrated the more classical pattern of nests and cords of uniform tumour cells floating in large mucin pools (Fig. 3). Cellular areas identical to those seen in the original biopsy could be found in the first recurrent tumour. Only a mucicarmine stain was available from this tumour that was intensely positive.

Case 2

An 80-year-old black male presented to Barnes Hospital in August, 1976, with a nodule on his right cheek which had been present for 20 years but had recently changed colour. It was described as non-tender, non-pigmented, and had three small telangiectases. A 7 mm cystic nodule was excised and diagnosed as mucinous carcinoma extending to the deep surgical margin. An extensive work-up in search of a primary mucinous tumour of internal organs failed to reveal evidence of another primary lesion. The work-up included: Intravenous pyelogram, chest-X-ray, metastatic bone survey, liver-spleen scan, barium enema and upper gastro intestinal series, thyroid scan, total body computerized tomography, and nasal sinus series. The patient is free of disease 8 months later.

Gross pathology: A 20×17×10 mm ellipse of skin overlying a 7 mm cystic nodule was described. On cut section the nodule had a glistening gelatinous appearance.

Microscopic examination. The tumour was composed of nests of uniform cells arranged in acini and cords immersed in large amounts of clear slightly basophilic amorphous mucin. These pools of mucin were divided into compartments by fibrous septa (Fig. 4). The epidermis was stretched and no ulcera-



FIGURE 1. Case 1, original tumour. Large nodules of tumour separated by fibrous septa. Note the striking resemblance to nodular hidradenoma (H & E, $\times 11$) (W.U. neg. 77-3897).

tion or connection with the tumour was seen. The cells showed a bland configuration (Fig. 5). No atypia or mitoses were observed. Sections stained for mucin including alcian blue (pH 2.5) mucicarmine, PAS, and colloidal iron were positive. The mucin was diastase and hyaluronidase resistant, and sialidase sensitive. Alcian blue stains at pH 1.0 (with and without hyaluronidase) were negative.

Case 3

A 63-year-old black male presented with a flesh coloured tumour on his right cheek. He was unable to state the duration of the lesion. It was non-tender. It was excised in another hospital and diagnosed as a sweat gland tumour with extensive mucin production. Four years later the patient presented at Barnes Hospital with a recurrence. The lesion was again excised and a diagnosis of mucinous carcinoma was made. The patient was lost for follow-up.

Gross pathology. The specimen was described as a jelly-like fragmented material measuring 7 mm in diameter.

Microscopic examination. The histology was identical to Case 2.

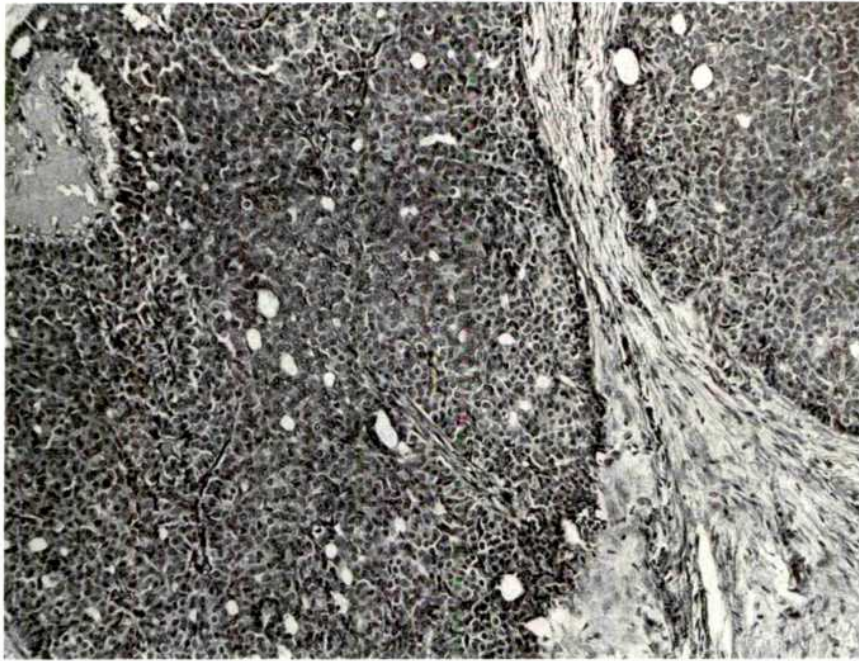


FIGURE 2. Case 1, original tumour. Solid sheets of tumour cells lining capillary septa. The small lumens contain mucin-positive proteinaceous material (H & E, $\times 150$) (W.U. neg. 77-4492).

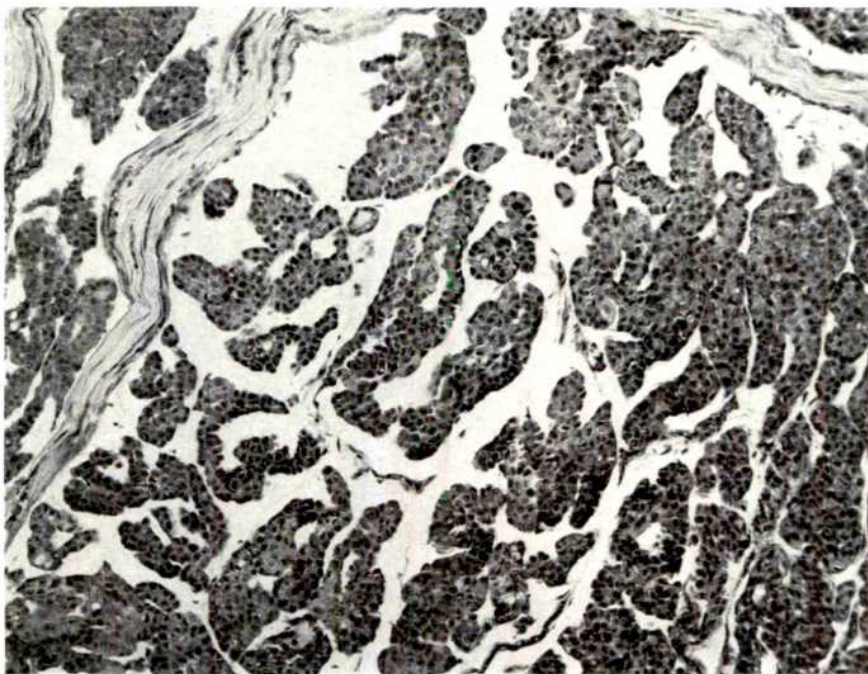


FIGURE 3. Case 1, recurrent tumour. There is an increase in the amount of intercellular mucins. Small lumens can still be distinguished (H & E, $\times 150$) (W.U. neg. 77-4493).

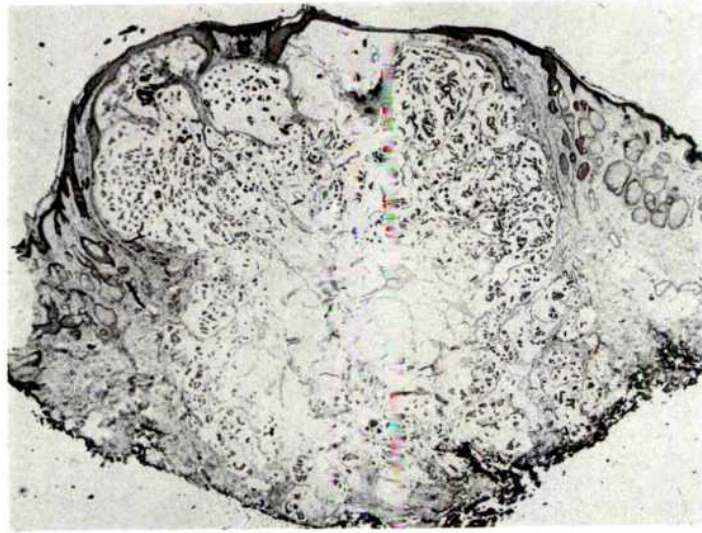


FIGURE 4. Case 2, A cross-sectional view of the entire tumour with overlying stretched epidermis. The resemblance to mucinous carcinoma of the breast is striking (H & E, $\times 11$) (W.U. neg. 77-3896).

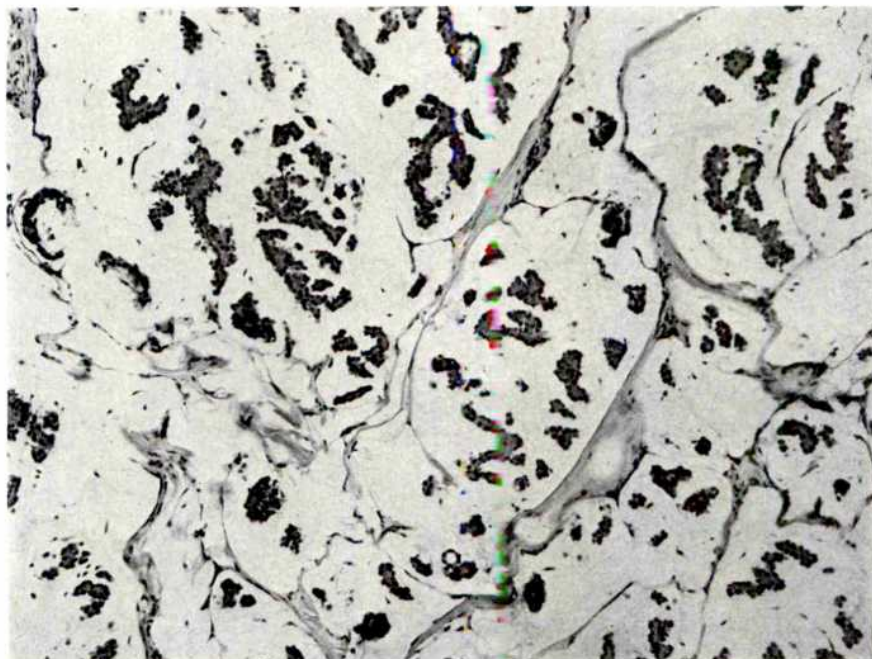


FIGURE 5. Case 2, Small epithelial islands floating in mucin pools separated by fibrous septa. Note the similarity to Fig. 2 (H & E, $\times 90$) (W.U. neg. 77-3893).

CLINICAL BEHAVIOUR

A review of the literature yielded thirty acceptable cases of primary mucinous carcinomas of the skin, including the three present cases. Published cases lacking adequate description or histological confirmation have not been included. A clinico-pathological profile can be established based on these cases. The general characteristics are presented in Table 1. The age was stated in 28 cases and ranged between 8 and 80 years; 24 were over the age of 50. Only five (17%) were women. The race was stated in 20 cases. Eleven were black, one was Japanese, and the remaining 8 cases were white. Twenty-two tumours were found on the head and neck, six were located in the axilla, two in the chest wall skin, and one each was found in the abdominal wall and foot. The size varied considerably with a range between 7 mm and 8 cm in greatest dimension. Fourteen were smaller than 2.5 cm. The dimensions were not reported in eleven. The largest tumour described was found on the abdominal wall and measured $8 \times 4 \times 2$ cm. Follow-up information was available on 17 patients. Ten patients have had recurrence between 8 months and 9 years later; three have had two recurrences. Four patients were free of recurrence. In spite of the high rate of recurrence, only three patients have had local lymph node metastases. One patient had metastases to scalp, face and several bones. The metastases were resistant to radiation therapy and differing alkylating agents.

Several points should be emphasized which are of clinical significance. The disease occurs most commonly in black, male, middle-aged, or elderly patients. There is no typical clinical presentation; most present as small, non-tender, long-standing nodules. There may be telangiectases over the lesion. A few cases showed ulceration. The recurrence rate is high and late recurrences are not unusual, but metastasis is rare.

DISCUSSION AND COMMENT

Mucin production has been seen in both the epidermis and its appendages in a variety of circumstances. Zak & Palladino (1969) commented on the normal occurrence of mucin-secreting cells in the integument of fishes and amphibians and in specialized cutaneous glands of some mammals. While mucin producing cells are not a normal feature of keratinizing epithelium, goblet cells occur normally in the conjunctiva. Lawrence & Bern (1966) have produced mucinous metaplasia in the hamster cheek pouch by treatment with vitamin A. Kwitten (1974) has reported an epidermal tumour containing goblet cells which he believed arose from metaplastic keratinocytes—an atavism. Zak & Palladino (1969) have noted an epidermal appendage tumour, classified as a solid-cystic hidradenoma, with goblet cells. Winkelmann & Hultin (1958) have noted apocrine gland mucinous metaplasia in 61 of 100 normal axillary skin specimens, and Lennox *et al.* (1952a) found mucinous material in both apocrine and eccrine glandular tumours. Mucin production may also be seen in association with hair follicles (alopecia mucinosa). In spite of the documentation of the ability of both the epidermis and its appendages to produce mucin, Lennox *et al.* (1952b) have proposed that all mucin producing tumours in the skin, with the exception of basal cell carcinoma, are of sweat gland origin. They have suggested that one tumour may secrete a variety of mucins and that epithelial mucin is modified by local factors to produce luminal mucins which in turn are modified to stromal and chondroid mucins. Their primary thesis was founded on this concept and the common histological pattern of secretory and myoepithelial layers in all mucin-producing tumours of the skin which they studied. Mendoza & Helwig (1971) have since demonstrated a non-sulphated acid mucopolysaccharide in primary mucinous carcinomas which differs from the mucin found in adenoid cystic carcinoma of lacrimal glands or chondroid syringomas, but which is similar to mucins found in both eccrine and apocrine sweat glands and

mucinous carcinoma of the breast (Johnson & Helwig, 1963). It was felt that these findings confirmed the sweat gland origin of these tumours although they were unable to differentiate between eccrine and apocrine origins. Headington (1977) has demonstrated a similarity between tumour cells and the dark secretory cells of the eccrine glands using electron microscopy and enzyme histochemistry. Cases of mucinous carcinoma of the skin previously published have been similar to our Cases 2 and 3 with nests and cords of uniform cells floating in mucin lakes.

Berg & McDivitt (1968) studied 102 sweat gland carcinomas and divided them into similar groups as their benign counterparts. Two of the nodular-hidradenomas-clear cell type-carcinomas had production of acid and neutral mucins. One classified as malignant papillary 'hidradenoma' had also large areas of acid mucin production. To our knowledge, a case demonstrating a transition from a solid, mucin-poor pattern resembling nodular hidradenoma to the typical pattern with large mucin lakes as seen in Case 1, has not been previously reported. Whether this pattern is present early in most tumours is not certain but Berg & McDivitt's (1968) experience seems to indicate so. It is suggested that lesions resembling hidradenoma be studied in greater detail in order to rule out a mucinous carcinoma. The end-stage pattern of these tumours is highly distinctive, and should suggest strongly its primary origin. However, a thorough search should be made to rule out a primary tumour elsewhere before the lesion is accepted as primary. The histological similarity to mucinous carcinomas of the breast is striking, but other visceral organs including gastrointestinal tract, lung, ovary and prostate should be investigated before considering a mucinous tumour primary in the skin. The importance of this differentiation cannot be too strongly emphasized in light of the relatively benign clinical behaviour of these lesions. Why these tumours behave differently from other sweat gland carcinomas which metastasize frequently is unclear, but Mendoza and Helwig (1971) have suggested that the uncontrolled mucin production of these relatively well-differentiated tumours interferes with cellular nutrition and thus limits the rate of growth. Headington (1977), using enzyme histochemistry, has demonstrated the high degree of differentiation of these tumours and postulated a DNA synthesis inhibition by these mucins as a result of cell product accumulation. They follow, therefore, the classical general rule in oncological pathology: well differentiated tumours have better prognosis than undifferentiated ones. Whatever the cause, the clinical behaviour is similar to that of basal cell carcinomas and adequate local excision seems to be the treatment of choice.

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REFERENCES

- BERG, J.W. & MCDIVITT, R.W. (1968) Pathology of sweat gland carcinoma. In: *Pathology Annual* (Ed. by S.C. Sommers), p.123. Appleton Century Crofts, New York.
- GROSSMAN, J.R. & IZUNO, G.T. (1974) Primary mucinous (adenocystic) carcinoma of the skin. *Archives of Dermatology*, **110**, 274.
- HEADINGTON, J.T. (1977) Primary mucinous carcinoma of skin, histochemistry and electron microscopy. *Cancer*, **39**, 1055.
- JOHNSON, W.C. & HELWIG, E.B. (1963) Histochemistry of the acid mucopoly-saccharides of skin in normal and in certain pathologic conditions. *American Journal of Clinical Pathology*, **40**, 123.
- KWITTEN, J. (1974) Muciparous epidermal tumor. *Archives of Dermatology*, **109**, 554.
- LAWRENCE, D.J. & BERN, H.A. (1960) Mucinous metaplasia and mucous gland formation in keratinized adult epithelium *in situ* treated with vitamin A. *Experimental Cell Research*, **16**, 443.
- LENNOX, B., PEARSE, A.G.E. & RICHARDS, H.G.H. (1952a) Mucin-secreting tumours of the skin: With special reference to the so-called mixed salivary tumour of the skin and its relations to hidradenoma. *Journal of Pathology and Bacteriology*, **64**, 865.

- LENNOX, B., PEARSE, A.G.E. & SYMMERS, W.St.C. (1952b) Frequency and significance of mucin in sweat gland tumours. *British Journal of Cancer*, **6**, 363.
- LUNA, L.G., editor (1968) *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd edn. Armed Forces Institute of Pathology, Washington DC.
- LUND, H.Z. (1957) Tumours of skin. In: *Atlas of Tumor Pathology*, Sect. 1—Fasc. 2, p. 120. Armed Forces Institute of Pathology, Washington.
- MENDOZA, S. & HELWIG, E.B. (1971) Mucinous (adenocystic) carcinoma of the skin. *Archives of Dermatology*, **103**, 68.
- METZ, J., FILIPP, N. & METZ, G. (1974) Mucinoses Carcinom der ekkrinen Schweissdrüsen. *Zeitschrift für Hautkrankheiten*, **49**, 963.
- MILLER, W.L. (1967) Sweat gland carcinoma. *American Journal of Clinical Pathology*, **47**, 767.
- RODRIGUES, M.M., LUBOWITZ, R.M. & SHANNON, G.M. (1973) Mucinous (adenocystic) carcinoma of the eyelid. *Archives of Ophthalmology*, **89**, 493.
- WINKELMANN, R.K. & HULTIN, J.V. (1958) Mucinous metaplasia in normal apocrine glands. *Archives of Dermatology*, **78**, 309.
- WOLFE, J.J. & STERNBERG, L.H. (1956) Metastasizing sweat gland carcinoma of the scalp involving transverse sinus. *American Journal of Surgery*, **88**, 849.
- ZAK, F.G. & PALLADINO, V.S. (1969) Muciparous metaplasia and primary mucoepidermoid skin tumor. *Archives of Dermatology*, **100**, 23.
- YEUNG, K-Y & STINSON, J.C. (1977) Mucinous (adenocystic) carcinoma of sweat glands with widespread metastasis. *Cancer*, **39**, 2556.

Relationship between T and B lymphocyte values and prognosis in malignant melanoma

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SUMMARY

T and B lymphocyte populations were evaluated in 56 patients with malignant melanoma. Active rosettes (T-Ea) were decreased only in metastatic patients, while the total T population (T-Et) was decreased in all stages. In addition the metastatic patients presented significant decreases in IgD and IgM subpopulations. An increase in null cells was noted in metastatic patients. Regular controls over a period of 2 years showed that T-Ea levels were closely linked to the clinical picture. Patients whose values were constant remained cancer free, while a reduction heralded the appearance of clinical and/or radiological signs of metastasis.

In the last few years there has been accumulating evidence that cancer patients have impaired cellular immunity of prognostic significance. In addition to skin tests, numerous *in vitro* assays have been developed for the assessment of cell-mediated immunity. The PHA test, however, is of little use in the vast majority of melanoma patients (Butterworth *et al.*, 1974), and the same is true of the PPD test. In mixed cultures, lymphocytes from stage II and stage III patients have displayed a low degree of immunogenicity. Rosette formation with sheep erythrocytes has been used to enumerate either the total circulating T-lymphocyte population (T-Et) (Jondal *et al.*, 1972) or to measure 'active T-lymphocytes' (T-Ea) (Wybran *et al.*, 1973). This test may be influenced by many pathological conditions. Nevertheless, the values obtained offer a general assessment of a patient's immune status. Fudenberg *et al.* (1975) have shown a close relationship between T-Ea and clinical status in cancer patients. The present paper reports the distribution of peripheral T and B lymphocytes in 56 malignant melanoma patients followed clinically and immunologically for 2 years.

MATERIALS AND METHODS

Patients. Twenty-five males and 31 females aged 23–81 years with histologically demonstrated malignant melanoma were studied. The classification of Lewis *et al.* (1969) was used to assign 28 patients to stage I, 3 to stage IIa, 15 to stage IIb, 7 to stage IIab and 3 to stage III. In 15 stage I

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subjects, immunological determinations were carried out at the time of diagnosis, before surgical removal of the lesion, and in 12 clinically cancer-free subjects from 15 days to 5 years after surgery. In the remaining stage I patient, the melanoma had been removed 11 years previously. Almost all subjects in stage II and III had been operated on for removal of the primary lesion. None had undergone lymph node resection when the first immunological tests were made. Neither chemotherapy nor immunotherapy had been attempted. All 56 patients were free from concomitant virus or bacterial diseases.

Immunological studies. E and EAC rosettes were determined according to the methods described by Jondal *et al.* (1972) and Bianco *et al.* (1970) as previously reported (Bernengo *et al.*, 1977). Active rosettes were determined according to Wybran's technique (1973). Surface immunoglobulins were determined in accordance with Pernis *et al.* (1970) using monovalent fluorescein-conjugated goat-anti-human immunoglobulin sera (anti IgG, IgA, IgM, IgE, IgD). The activity and specificity of these antisera were tested by immunodiffusion using the appropriate purified human antigens. Fluorescence was read with a Leitz microscope equipped with a mercury vapour lamp and fluorescence vertical illumination.

RESULTS

Absolute and percentage T-Ea, T-Et, EAC rosette and null cell values for patients in stage I are given in Table 1, along with lymphocyte levels in 24 normal subjects. T-Ea values were within the normal range, whereas there was a significant reduction in T-Et values. EAC rosettes were not significantly different from the control levels. Null cells were increased in percentage, though there was no significant difference in their absolute values. Table 2 presents T and B lymphocyte values for patients with metastases. Here T-Ea values were significantly reduced and T-Et levels were lower than in stage I. EAC levels were unchanged, while null cells showed a significant increase ($P < 0.001$).

Surface immunoglobulins (SmIg) in 25/28 stage I patients are shown in Table 3. IgD only displayed a significant fall in comparison with normal controls. The metastatic patients, on the other hand, showed significant decreases in both IgD and IgM when compared with controls (Table 4).

All patients were followed immunologically every 3 months for 2 years. Serial rosette values were normal in 18 stage I patients who had been clinically cancer-free for 2 years.

Four months after surgery, one patient (no. 18) presented with a subcutaneous nodule on the same limb. Her T-Ea and T-Et values fell from 18.5 to 11% and from 49.5 to 37.5% respectively. After surgical removal of this histologically proven metastasis, these values rose to 34 and 60%. Ten months later, they fell again to 10 and 37%, followed after a further 2 months by inguinal adenopathy.

Patient no. 26 remained cancer-free for 1 year with stable, normal T-Ea and T-Et. In October 1976, they fell from 19 to 11% and from 56 to 39.5% respectively and by January 1977 T-Ea had dropped to 6.5%. Lymphography revealed signs of secondary involvement at this time.

High T-Ea and T-Et levels were maintained for 14 months by patient no. 11. Axillary node enlargement was observed 3 months after falls to 18.5 and 39.5% respectively.

Sudden falls of T-Ea to 13% and T-Et to 18% occurred after 1 year in Case no. 7. No adenopathy could be detected clinically and the lymphography picture was negative. After 3 months at these levels, inguinal node involvement was noted. Surgery revealed extensive invasion of the deep lymph nodes.

Patients 5, 9 and 24 have no clinical or radiological signs of metastasis at present. However their,

TABLE 1. Rosette formation with lymphocytes from melanoma patients in stage I

Patients no.	% TE _a	% TE _b	% EAC	% Null cells	Absolute lymphocyte count/mm ³	Absolute TE _a /mm ³	Absolute TE _b /mm ³	Absolute EAC/mm ³	Absolute Null cells/mm ³
1	22.5	49	28.5	22.5	2430	547	1191	692	547
2	30.5	45	11	44	1824	556	821	201	802
3	37.5	60	10	30	2480	930	1488	248	744
4	26	65	35	0	1755	456	1140	615	0
5	35.5	49	14.5	36.5	2538	901	1244	368	926
6	26.5	41	24	35	2546	675	1044	611	891
7	32.5	54.5	20	25.5	1472	478	802	295	375
8	41.5	60.5	19	20.5	2494	1035	1509	474	511
9	40.5	42.5	29	28.5	1785	723	759	517	509
10	44	55	21.5	23.5	1400	616	770	301	329
11	30	73	15.5	11.5	1739	522	1270	269	200
12	30.5	54.5	10	35.5	1440	439	785	144	511
13	19.5	67	12	21	3430	669	2298	412	720
14	21.5	34	11.5	54.5	2320	499	789	267	1264
15	37.5	44	20	36	1456	546	641	291	524
16	22	57	22	21	1596	351	910	351	335
17	32.5	62	22	16	2201	715	1365	484	352
18	18.5	49.5	15.5	31	2332	431	1154	455	723
19	24	53	15.5	31.5	1479	355	784	229	466
20	38	70	8	22	1813	689	1269	145	399
21	40.5	68.5	26	5.5	1624	658	1113	422	89
22	17	51	33	16	2016	343	1028	665	323
23	22.5	60	13	27	1645	370	987	214	444
24	17.5	61.5	9.5	29	2835	496	1743	270	822
25	34	79	17	4	1140	387	900	194	46
26	19.5	54	22	24	2040	398	1102	449	489
27	23.5	61.5	27.5	11	3276	770	2015	901	360
28	33.5	45	23	32	1917	642	863	441	613
Mean $\pm$ s.d.	29.2 $\pm$ 8.1	55.9 $\pm$ 10.5	19.2 $\pm$ 7.3	24.4 $\pm$ 11.6	2036 $\pm$ 570	579 $\pm$ 183	1135 $\pm$ 393	390 $\pm$ 182.1	511 $\pm$ 291.3
Healthy controls	31.9 $\pm$ 8.9	64.7 $\pm$ 7.6	17.5 $\pm$ 9.3	17.6 $\pm$ 10.5	2425 $\pm$ 706	727 $\pm$ 277	1543 $\pm$ 519	463 $\pm$ 218	419 $\pm$ 263
Student's <i>t</i> -test	N.S.	$P < 0.002$	N.S.	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.005$	N.S.	N.S.

TABLE 2. T and B cells in peripheral blood of melanoma patients in stage II and III

Patient no.	%TE _a	%TE _i	%EAC	% Null cells	Absolute lymphocyte count/mm ³	Absolute TE _a /mm ³	Absolute TE _i /mm ³	Absolute EAC/mm ³	Absolute Null cells/mm ³
1	22.5	52	14.5	33.5	1260	283	655	183	422
2	4.5	20	23.5	56.5	2064	92	412	485	1167
3	15	21.5	11.5	67	2144	321	461	246	1437
4	10	60.5	10.5	29	1612	161	975	169	468
5	12.5	40.5	22.5	37	1464	183	593	329	542
6	17	18.5	15	66.5	1400	238	259	210	931
7	5.5	18	17.5	64.5	2304	126	415	403	1486
8	19.5	18.5	27	54.5	2419	472	448	653	1318
9	15.5	25.5	13.5	61	2666	413	680	360	1626
10	11.5	51	20	29	1482	170	756	296	430
11	16	62.5	5	32.5	2448	392	1530	122	796
12	4.5	11.5	18	70.5	2546	114	293	458	1795
13	16	54	25.5	20.5	1200	192	648	306	246
14	3.5	13	11	76	1350	47	175	149	1026
15	12.5	56.5	26	17.5	3224	403	1822	838	564
16	16	37.5	12	50.5	2550	408	956	306	1288
17	34	40	16.5	43.5	2905	987	1162	479	1264
18	17	28.5	7	64.5	1037	176	295	73	669
19	9.5	31.5	7.5	61	1363	129	429	102	832
20	4	15.5	18	66.5	1904	76	295	343	1266
21	9.5	52	23	25	1995	189	1037	459	499
22	8.5	37	29	34	1800	153	666	522	612
23	11	55.5	16	28.5	2183	240	1212	349	622
24	2.5	38	14.5	47.5	4845	125	1841	703	2302
25	7.5	58.5	30	11.5	3276	245	1916	983	377
26	4	47	24	29	2117	84	995	508	614
27	11	50	14	36	2320	255	1160	325	835
28	10.5	33	20	47	2184	229	721	437	1026
Mean $\pm$ s.d.	11.82 $\pm$ 6.85	37.41 $\pm$ 16.24	17.59 $\pm$ 6.78	45 $\pm$ 18.29	2145.07 $\pm$ 798.8	246.4 $\pm$ 185.0	814.5 $\pm$ 499.3	385.58 $\pm$ 217.1	945 $\pm$ 495.1
Healthy controls (24)	31.9 $\pm$ 8.9	64.7 $\pm$ 7.6	17.5 $\pm$ 9.3	17.6 $\pm$ 10.5	2425 $\pm$ 796	727 $\pm$ 277	1543 $\pm$ 519	463 $\pm$ 218	419 $\pm$ 263
Student's <i>t</i> -test	$P < 0.001$	$P < 0.001$	N.S.	$P < 0.001$	N.S.	$P < 0.001$	$P < 0.001$	N.S.	$P < 0.001$

TABLE 3. Sm Ig positive cells in peripheral blood of melanoma patients in stage I

Patient no.	% Sm Ig				
	IgG	IgA	IgM	IgE	IgD
1	4.5	1	5.5	1.5	8.5
3	10.5	3	8.5	2	3
4	7	9.5	8.5	1	2.5
5	4.5	5	8.5	1	7
6	6	3.5	1.5	1.5	4
7	8	4	6.5	1	4
8	6.5	8	7	1	2
9	3	0.5	1	0.5	1
11	8	1.5	4.5	0	0.5
12	3.5	1	5	0	0
13	3	1.5	2	0.5	0
14	0.5	1.5	2.5	0.5	0
15	6.5	2	11	0.5	3.5
16	1	0	6	—	—
17	2.5	1.5	3.5	0	1.5
18	7.5	1	3	1	2.5
19	9	2	2.5	1.5	2
20	5	1	0.5	0.5	1.5
21	3	3	3.5	2.5	1.5
22	6	2.5	6.5	0.5	4
23	3.5	4	3	0.5	4
24	2.5	0.5	5	1	2.5
26	11	13	9	2	6
27	5.5	3.5	3.5	1	2
28	8	2	16	5	7
Mean $\pm$ s.d.	5.4 $\pm$ 2.8	3.0 $\pm$ 3.0	5.4 $\pm$ 3.5	1.1 $\pm$ 1.1	2.9 $\pm$ 2.3
Healthy controls (26)	6.4 $\pm$ 3.4	3.0 $\pm$ 1.6	6.8 $\pm$ 1.9	0.8 $\pm$ 0.8	4.3 $\pm$ 1.5
Student's 't'-test	N.S.	N.S.	N.S.	N.S.	$P < 0.02$

T-Ea and T-Et values have declined from normal to low levels and they will be closely watched for signs of relapse.

Nine stage II and III patients (nos 2, 3, 5, 6, 7, 12, 13, 14, 20) with very low T-Ea and T-Et levels, particularly their absolute values, displayed visceral metastases and died within a few months.

Patients with normal T-Et, on the other hand, are still in good general health 18 months later, despite the appearance of further skin metastases (nos 11, 15, 17, 27).

Patient P.V. (no. 1, Table 4) appeared to be in stage I when first seen: T-Ea and T-Et were 22 and 52%, though their absolute values were well down at 283 and 655/mm³ respectively. Nine months later, inguinal edanopathy was noted (T-Ea=8% and T-Et=61%). After a further 3 months, values had fallen to T-Ea 4.5% and T-Et 50.5% with 1300 lymphocytes/mm³. Disseminated skin and lung metastases were apparent and death due to liver metastasis soon followed.

TABLE 4. Sm Ig positive cells in peripheral blood of melanoma patients in stages II and III

Patient no.	% Sm Ig				
	IgG	IgA	IgM	IgE	IgD
1	5.5	1.5	1.5	1	0.5
3	7	3.5	3	2	5
4	7	5	12	2.5	1.5
5	7	6	4	1	3
6	8	6	11	0	6
7	8	0.5	2	0	3
8	2.5	2.5	4	1.5	1.5
9	11	6	3	1	4
10	12	1.5	6.5	1	2
11	6.5	4.5	5.5	1	-
15	13	1.5	3.5	0.5	5
16	9.5	3.5	5.5	0	2.5
17	3	2.5	1	0	1
18	0.5	-	0.5	0	1.5
19	3	-	3	1	0.5
20	7	1	1	0	5
21	4	3	0.5	0	0.5
23	1	7.5	2	0	3.5
26	3	2	1.5	1	1.5
27	6.5	5.5	2	1	4.5
28	9.5	3.5	9.5	2.5	
Mean $\pm$ s.d.	6.4 $\pm$ 3.5	3.5 $\pm$ 2.0	2.9 $\pm$ 3.3	0.8 $\pm$ 0.8	2.7 $\pm$ 1.8
Healthy controls (26)	6.4 $\pm$ 3.4	3.0 $\pm$ 1.6	6.8 $\pm$ 1.9	0.8 $\pm$ 0.8	4.3 $\pm$ 1.5
Student's 't'-test	N.S.	N.S.	$P < 0.001$	N.S.	$P < 0.005$

DISCUSSION

A significant and interesting connection between immunological status and clinical stage was noted in 56 patients with malignant melanoma followed for 2 years. In stage I subjects, T-Et values were well down, as reported by Koziner, Cosimi & Bloch (1975). This depression may be related to the presence of small tumour deposits. Fifteen of our subjects were tested before ablation of the melanoma; 9 displayed decreased T-Et values followed by a return to normal levels 1 month after surgery. An alternative explanation is that receptors for sheep erythrocytes were coated with blocking substances in some of our patients. Whitehead *et al.* (1976) found that papain treatment of lymphocytes from breast cancer patients increased the number of E-rosettes. Similar results were obtained by Rugarli *et al.* (1975) after prolonged washing. However, we observed no difference in the ability to form rosettes after prolonged washing. We did not employ papain.

A decrease in T cell percentages has been reported in older subjects. Our findings, however, disclose no relation between T lymphocyte level and age. Patients over 50 years who were cancer free for 2 years after surgery displayed constant and normal values. T-Ea have been seen as a subpopulation more actively engaged in cell immunity than the T-Et. Wybran & Fudenberg (1973) found low levels and a close relation between T-Ea number and stage in cancer. Claudy *et al.* (1975) observed a poor

prognosis in 2 of 16 melanoma patients with actinic rosette values of less than 15%. We found normal T-Ea values in our stage I patients. These remained constant when no signs of metastasis appeared, whereas a fall in value was sometimes noted a few months before clinical evidence of metastasis was obtained. It would seem that T-Ea values are not influenced by the presence of small tumour deposits. Indeed, no difference in T-Ea was noted between those who had and those who had not been treated surgically. Metastases, however, were accompanied by a fall in T-Ea with respect to both stage I patients and the controls. No relation was observed between E-rosette values and the level of melanoma invasion, nor between rosette values and tumour site.

Contrary to the findings of Malka *et al.* (1974), we did not observe an increase in IgD bearing lymphocytes. Indeed, metastatic patients displayed a significant fall in both IgD and IgM. Thivolet *et al.* (1977) reported an absence of plasma cells secreting IgM in the peritumoural infiltrate of melanoma.

It would appear, therefore, that evaluation of the cell-mediated immunological situation is of prognostic value in malignant melanoma. Periodic controls also serve as a guide in the choice of chemotherapeutic and/or immunotherapeutic protocols.

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REFERENCES

- BERNENGO, M.G., CAPELLA, G., MASCOLI, I. & ZINA, G. (1977) Active rosette test in cutaneous lymphoproliferative disorders. *Dermatologica*, **154**, 342.
- BIANCO, C., PATRICK, R. & NUSSENZWEIG, V. (1970) A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. *Journal of Experimental Medicine*, **132**, 702.
- BUSTAMANTE, R., SCHMITT, D., PILLET, C. & THIVOLET, J. (1977) Immunoglobulin-producing cells in the inflammatory infiltrates of cutaneous tumours. Immunocytologic identification *in situ*. *Journal of Investigative Dermatology*, **68**, 346.
- BUTTERWORTH, C., OON, C.J., WESTBURY, G. & HOBBS, J.R. (1974) T-lymphocyte responses in patients with malignant melanoma. *European Journal of Cancer*, **10**, 639.
- CLARK, W.H., Jr, FROM, L., BERNARDINO, E.A. & MIHM, M.C., Jr (1969) The histogenesis and biologic behaviour of primary human malignant melanomas of the skin. *Cancer Research*, **29**, 705.
- CLAUDY, A., FOUAD-WASSEF, N. & PELLETIER, N. (1975) Valeur du test des rosettes thymodependantes dans la surveillance des melanomes malins. Etude préliminaire à propos de 25 cas. *Annales de Dermatologie et de Syphiligraphie*, **102**, 69.
- FUDENBERG, H.H., WYBRAN, J. & ROBBINS, D. (1975) T-rosette forming cells, cellular immunity and cancer. *New England Journal of Medicine*, **27**, 475.
- JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. *Journal of Experimental Medicine*, **136**, 207.
- KOZINER, B., COSIMI, A.B. & BLOCH, K.J. (1975) Distribution of latex-ingesting cells, T cells and B cells in the peripheral blood of patients with malignant melanoma. *Journal of National Cancer Institute*, **55**, 1295.
- LEWIS, M.G., IKONOPISOV, R.L., NAIRN, R.C., PHILLIPS, T.M., HAMILTON-FAIRLEY, G., BODENHAM, D.C. & ALEXANDER, P. (1969) Tumour specific antibodies in human malignant melanoma and their relationship to the extent of the disease. *British Medical Journal*, **iii**, 547.
- MALKA, S., OON, C.J. & HOBBS, J.R. (1974) Excess IgD bearing lymphocytes in patients with malignant melanoma. *British Journal of Cancer*, **30**, 379.

Melanoma specific protein: occurrence in the urine of patients with halo naevus and vitiligo

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SUMMARY

Melanoma specific protein is immunologically related to altered naevus cell cytoplasm. It is excreted by patients with malignant melanoma but in no other malignancy. The protein has been detected in patients with actively developing halo naevi but not when repigmentation is taking place. It also occurs in patients with very active vitiligo but in no other pigment condition we have studied. It is suggested that the protein is a marker of active destruction of naevus cells by immune mechanisms and that the release of toxic materials during this immune reaction may be responsible for the production of the halo phenomenon and for the areas of vitiligo that may be seen elsewhere on the skin.

Two separate groups of workers, Jehn *et al.* (1970) and Carrel & Theilkaes (1973) have described the detection of a tumour specific protein in the urine of patients with malignant melanoma. Precipitating antisera were raised in rabbits by injecting urine concentrates and after absorption were used to detect the tumour associated antigen in urine by double diffusion in agar gel. We have raised a non-precipitating antiserum in rabbits using malignant melanoma cells, and have used this antiserum in an antibody absorption procedure (Bennett, Cooke & Geck, 1976) to test the urines of patients with malignant melanoma (Bennett *et al.*, 1977). No malignant disease so far tested has shown a cross-reacting urine protein. Urine from a patient with neuroblastoma, however, contained a similar protein reacting specifically with anti-neuroblastoma antibody.

Because of this unique specificity with individual malignant conditions we decided to examine urine from patients with non-malignant pigment disease, where destruction of melanocytes or naevi was occurring. The self-healing melanocytic mole, the halo naevus, has been reported to be immunologically related to malignant melanoma, with antibodies present in the patient's serum which react with the cytoplasm of malignant melanoma cells (Copeman, *et al.*, 1973). In this paper we report that the urines of patients with actively developing halo naevi similarly contain a protein that can inhibit the cytoplasmic staining of malignant melanoma cells by a specific rabbit anti-human melanoma antiserum.

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MATERIALS AND METHODS

Heterologous anti-human melanoma antiserum (R.A.M.A.) was raised in rabbits using neuraminidase-treated stored fresh-frozen tumour cells according to the method of Ray, Thakur & Sunduran (1975).

The harvested antiserum was absorbed with pooled human red blood cells, pooled human lymphocytes (23 patients), normal skin, normal organs (heart, kidney and lung), insolubilized human serum proteins (Avrameas & Ternynck, 1969) and insolubilized urine solids (Bennett *et al.*, 1976) until no further cross reactions could be demonstrated. This absorbed antiserum still gave positive immune fluorescence with normal naevus tissue and further absorptions with normal mole were performed to remove this activity.

When the indirect immunofluorescence method and strict criteria of Elliott *et al.* (1973) were applied the final preparation gave positive cytoplasmic staining with human melanoma cells and cells from a halo naevus. No staining was seen with any other tumour tested (carcinoma of breast, colon, mandible, tongue, basal cell carcinoma) nor with either stored or cultured cells from the embryologically related neuroblastoma. The antiserum did not precipitate soluble melanoma antigens in the double diffusion technique and did not agglutinate sheep red blood cells coated with soluble melanoma antigen.

In the indirect fluorescence test it titred at 1:25.

Urine protein. Random urine samples were dialysed against 10 vols distilled water overnight at 4°C with continuous stirring. Protein concentration was measured on the dialysed urine using a modified Folin-Lowry technique (Hartree, 1972).

Melanoma specific protein was detected by a fluorescence inhibition procedure (Bennett *et al.*, 1976) in which a volume of dialysed urine containing 20 mg of protein was insolubilized onto 500 mg AH-Sepharose 4B beads (Pharmacia Ltd.) by reaction with 20 mg 1-ethyl-3-(3 dimethylamino-propyl) carbodiimide HCl at pH 5.2-5.5 overnight at 4°C. After suitable washings to remove unreacted proteins and reagents the beads were resuspended in 0.5 ml dilute R.A.M.A. (1 ml R.A.M.A. + 7 ml P.B.S.) at room temperature for 60 min at neutral pH on a Matburn mixer. The beads were separated on a Buchner Funnel and the absorbed antiserum (filtrate) used to stain the cytoplasm of snap-frozen human melanoma cells (previously stored fresh-frozen). Visualization of residual antibody activity was by the indirect immunofluorescence technique using a fluorescein labelled goat anti-rabbit globulin antiserum (Hoechst Pharmaceuticals Ltd.) diluted with phosphate buffered saline (1 ml antiserum + 9 ml P.B.S.). Less than 25% cells showing cytoplasmic staining was taken as negative fluorescence and hence indicated the presence of melanoma specific protein in the original urine.

PATIENTS STUDIED

Patients with halo naevus

Thirteen patients have been studied, of whom 7 were male; 5 were under 20 years of age. Five female and two male patients each had a solitary halo naevus. In 11 patients the naevi were reported as still active at the time of urine collection and with three of these patients further specimens were obtained after repigmentation had commenced. Two patients (Staughton *et al.*, 1977), mother and son, presented with a familial form of the condition. One patient had Crohn's disease, which had been treated surgically. Four had associated vitiligo.

Other patients

Eleven patients with vitiligo, four patients with other non-malignant naevi (two with junctional naevi, one with a blue naevus, and one case of juvenile melanoma: all removed at biopsy), one patient with deeply pigmented seborrhoeic warts, one patient with morphea and one patient with pityriasis alba were tested in the same series.

Normal urine samples were provided by laboratory staff (22 male; 2 female) in the age range 18–48 years. The group consisted of 16 Caucasians, 2 Negroes, 3 Indians and 3 Chinese.

RESULTS

The results of urine tests on 140 patients treated for malignant melanoma have been reported previously (Bennett *et al.*, 1977) and show an increasing incidence of melanoma specific protein from 50% occurrence in patients with isolated primary tumours (Stage I) to 83% occurrence in patients with widespread metastases (Stage III). The results did show an anomaly in that 44% of patients who had survived more than 5 years excreted melanoma specific protein, and this may represent symbiosis between patient and tumour (Volkers *et al.*, 1977).

TABLE 1. Detection of melanoma specific protein in the urine of 31 patients with assorted pigment conditions

Clinical conditions	Urinary MSP present
Halo naevus	
Active solitary lesion	6/7
Active multiple naevi	3/5
Repigmenting multiple naevi	1/4
Other pigmentary conditions	
Vitiligo active	3/5
inactive	1/6
Others*	0/7
Normals	0/24

* Blue naevus (1), juvenile melanoma (1), junctional naevus (2), localized morphea (1), pityriasis alba (1), seborrhoeic warts (1).

The results of our studies on the 31 patients listed above are shown in Table 1 and these show that 9 of 12 patients with actively progressing halo naevi excreted specific protein but that this excretion ceased when naevus destruction stopped. Likewise 3 of 5 patients with very active vitiligo excreted specific protein but in only 1 of 5 patients with inactive vitiligo was the protein detected. No specific protein was found in the urine of any of the normals or any other patient examined. Of the four patients with multiple repigmenting haloes three had been studied earlier when the naevi were active and had been excreting melanoma specific protein at that time. Neither the patient with a solitary halo naevus nor the two patients with multiple halo naevi, but without urine protein, were biopsied. The clinical histories of all these patients indicated that the haloes had persisted for more than a year so that it is highly likely that the naevi were no longer active.

DISCUSSION

The occurrence in human urine of proteins related to the cytoplasmic antigen of malignant melanoma

is now well established (Jehn *et al.*, 1970; Carrel & Theilkaes, 1973; Bennett *et al.*, 1976). Unlike α -fetoprotein and carcino-embryonic antigen, which can be detected in many varied malignancies (for review see Schwartz, 1976), melanoma specific protein has proved to be truly tumour specific, occurring uniquely in patients with malignant melanoma of all the cancer patients tested. In this respect it resembles the pancreatic onco-fetal antigen of Banwo, Versey & Hobbs (1973), the nephroblastoma antigen (Jalanko & Ruoslahti, 1976) and the neuroblastoma antigen which we have described (Bennett *et al.*, 1976). The detection of this protein in the urine of patients with the benign condition of halo naevus was therefore surprising and raises, once again, the question of a link between the destructive processes in the two conditions.

Copeman *et al.* (1973) have shown that antimelanoma cytoplasm antibody could be detected in the sera of patients with halo naevi during the inflammatory stage but that this disappeared when the lymphocytic infiltrate disappeared. They suggested that halo naevus represented an exaggerated form of natural protection, with massive and rapid rejection of the whole naevus tumour mass, whilst malignant melanoma, particularly with metastasing tumour, represented unsuccessful rejection. In their view the destruction of normal skin melanocytes in the overlying epidermis and in the remote areas of associated vitiligo were part of the same immunological phenomenon.

In our hands melanoma specific protein is detected with an antibody that does not react with either normal skin or normal naevus. If the same mechanism is involved in the destruction of 'tumour' (i.e. naevus cell) and of 'peri-tumour' area (i.e. halo) then it would appear to result in immunologically distinct processes in the two situations. Neither autologous melanoma antibody nor autologous halo naevus antibody react with either normal skin or normal mole and we feel that it is unlikely that the same immunological mechanism is involved in both naevus cell and epidermal melanocyte destruction or that the immunological theory can satisfactorily explain the production of the halo. Moreover, with one exception (Langhof, Feuerstein & Schabinski 1965), immunological mechanisms have not been demonstrated in vitiligo. Our detection of melanoma specific protein only in the most active phases of this disease would indicate that immune reactions play only a transient role at the onset and that other mechanisms are responsible for maintaining the disease.

A likely explanation is that the specific immunological reaction, or its by-products, triggers the natural destructive processes responsible for vitiligo. Lerner (1971) proposed that melanocytes do not detoxicate melanogen precursors efficiently and his self-destructive theory, to account for greying hair, suggested that the gradual build up of these toxic precursors eventually caused cell death. In the condition of halo naevus massive naevus cell death would lead to the release of such precursors and hence to the death of surrounding melanocytes. In such a system a self-perpetuating diffusion gradient of toxin would cause a slow outward spread of the halo. Logically such a mechanism should also operate in malignant melanoma although reports of haloes in this disease are rare (Copeman *et al.*, 1973). Possibly in malignant melanoma the outward spread of the growing tumour into the potential halo region masks chemical destruction of melanocytes in the surrounding skin.

Chemical changes in the areas adjacent to a tumour are well established and colonic tumours, in particular, have been intensively investigated and form a good model for our propositions. Felipe (1969) described three areas; (i) normal mucosa (distant from the tumour); (ii) transitional mucosa (the peritumour area); (iii) tumour. Distinct, characteristic differences in hexosamines and sialic acids between these three areas have been shown (Felipe & Cooke, 1974). Interestingly similar changes have been observed in familial polyposis and the analogy becomes even closer when the 'Swiss roll' technique of histological examination is used on the excised colon. With this technique Felipe & Branfoot (1975) were able to demonstrate the histopathological changes consistent with transitional mucosa in non-tumorous foci distant from the tumour site. Thus in the colonic carcinoma model we see the analogy of the three situations (a tumour with induced secondary changes in the

peritumour area; an analogous pattern surrounding an associated non-malignant condition; and areas of transitional mucosa remote from the tumour) which we present here as malignant melanoma, halo, and associated distant vitiligo.

We would, therefore, propose that the changes seen in malignant melanoma and halo naevus are initiated by a common immunological response to altered naevus cells and that the by-products of this response can induce the histopathological changes of vitiligo in the halo through the mechanism proposed by Lerner (1971). With this proposition, the immunological theory of naevus destruction still remains central to an understanding of naevus cell death but it is no longer necessary to extend the theory to encompass the vitiliginous changes of the surrounding halo, where immune reactions have never been successfully demonstrated.

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REFERENCES

- AVRAMEAS, S. & TERNYNCK, T. (1969) The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoabsorbents. *Immunochemistry*, **6**, 53.
- BANWO, O., VERSEY, J. & HOBBS, J.R. (1973) New onco-fetal antigen for human pancreas. *Lancet*, **ii**, 643.
- BENNETT, C., COOKE, K.B. & GECK, P. (1976) Protein insolubilization as an aid to the detection of melanoma antigen in human urine. *Protides of Biological Fluids*, **24**, 667.
- BENNETT, C., COOKE, K.B., STAUGHTON, R.C.D., ELLIOTT, P.G., VOLKERS, C. & HOBBS, J.R. (1978) The occurrence and specificity of melanoma antigen. *Proceedings of the European Association of Cancer Research Meeting*, **4**, in press.
- CARREL, S. & THEILKAES, L. (1973) Evidence for a tumour-associated antigen in human malignant melanoma. *Nature*, **242**, 809.
- COPEMAN, P.W.M., LEWIS, M.G., PHILLIPS, T.M. & ELLIOTT, P.G. (1973) Immunological associations of the halo naevus with cutaneous malignant melanoma. *British Journal of Dermatology*, **88**, 127.
- ELLIOTT, P.G., THURLOW, B., NEEDHAM, P.R.G. & LEWIS, M.G. (1973) The specificity of the cytoplasmic antigen in human malignant melanoma. *European Journal of Cancer*, **9**, 607.
- FELIPE, M.I. (1969) Value of histochemical reactions for mucosubstances in the diagnosis of certain pathological conditions of the colon and rectum. *Gut*, **10**, 577.
- FELIPE, M.I. & COOKE, K.B. (1974) Changes in the composition of mucin in the mucosa adjacent to carcinoma of the colon as compared with the normal: a biochemical investigation. *Journal of Clinical Pathology*, **27**, 315.
- FELIPE, M.I. & BRANFOT, A.C. (1974) Abnormal patterns of mucous secretion in apparently normal mucosa of large intestine with carcinoma. *Cancer*, **34**, 282.
- HARTREE, E.F. (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Annals of Biochemistry and Experimental Medicine*, **48**, 422.
- JEHN, U.W., NATHANSON, L., SCHWARTZ, R.S. & SKINNER, M. (1970) *In vitro* lymphocyte stimulation by a soluble antigen from malignant melanoma. *New England Journal of Medicine*, **283**, 329.
- JALANKO, H. & RUOSLAHTI, E. (1976) Lack of human material inhibitory in fetuin radioimmunoassay. Contribution of carbohydrate moiety to fetuin antigenity. *Protides of Biological Fluids*, **24**, 303.

- LANGHOF, H., FEUERSTEIN, M. & SCHABINSKI, G. (1965) Melanin antibody formation in vitiligo. *Hautarzt*, **16**, 209.
- LERNER, A.B. (1971) On the aetiology of vitiligo and grey hair. *American Journal of Medicine*, **51**, 141.
- RAY, P.K., THAKUR, V.S. & SUNDARAM, K. (1975) Antitumour immunity. (1) Neuraminidase-treated and X-irradiated tumour vaccine. *European Journal of Cancer*, **11**, 1.
- SCHWARTZ, M.K. (1976) Detection of tumour associated antigens in plasma or serum. *Clinical Immunobiology*, **3**, 405.
- STAUGHTON, R.C.D., COPEMAN, P.W.M., COOKE, K.B. & BENNETT, C. (1978) Case Report. *Proceedings of the Royal Society of Medicine*, in press.
- VOLKERS, C., COOKE, K.B., BENNETT, C., BYROM, N., ELLIOTT, P.G., WHITFIELD, P. & EARLE, J.H.O. (1978) The significance of urinary melanoma antigen excretion and the ability of thymosin to raise the level of depleted T lymphocytes *in vitro* in melanoma. *Australian and New Zealand Journal of Surgery*, in press.

'Fiddler's neck'

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SUMMARY

'Fiddler's neck' is a condition affecting violin and viola players. Although well known to musicians it is not well recognized by dermatologists. Clinically the lesions usually consist of a localized area of lichenification on the left side of the neck—just below the angle of the jaw. Pigmentation, erythema and inflammatory papules or pustules are frequently present, while severe inflammatory induration, cyst formation and scarring occur in more severely affected subjects. The aetiology of the skin changes is probably due to a combination of factors; friction giving rise to lichenification, while local pressure, shearing stress and occlusion may play a part in producing the acne-like changes and cyst formation. In addition, poor hygiene may predispose to local sepsis.

Characteristic occupational marks occur in many different trades and professions (Ronchese, 1948). A number of such marks have been noted among musicians and include calluses on the finger tips of violinists and other string instrumentalists, localized thickenings on the lips in woodwind players, and looseness of the cheeks in tuba players. A common dermatological problem in professional and student violinists is 'fiddler's neck'—a condition in which an area of redness, thickening and inflammation of the skin occurs under the angle of the left side of the jaw—where the chin rest of the violin is in contact with the side of the neck. Although well recognized by violinists and viola players, the condition is not often brought to the attention of the dermatologist who may, therefore, be unfamiliar with it.

Our interest in 'fiddler's neck' was aroused when a professional violinist, who had previously been under the care of Dr Grant Peterkin and Dr Mary Bunney in Edinburgh, moved to Bristol and was referred to out-patients. The present study was carried out in order to determine the causes and clinical features of this condition.

SUBJECTS AND METHODS

Our original patient put us in contact with two of her pupils who had 'fiddler's neck'. One pupil, a member of the Academy of the B.B.C. Orchestra, arranged with the leader of the orchestra for us to meet, examine and photograph a further nine affected violin and viola players. All affected subjects filled in a questionnaire, which included details of the instrument played, age of starting to play and of the onset of 'fiddler's neck', total hours of daily practice, clinical features and the factors that seemed

to make the condition better or worse. At our request, the Lady Superintendent of the Royal Academy of Music kindly arranged for us to examine and photograph a further ten affected students of the violin and viola, who also filled in our questionnaire.

RESULTS

A total of 23 subjects (14 male, 9 female) with 'fiddler's neck', whose ages ranged from 17 to 58 years, filled in the questionnaire. Twenty-two of these were examined and photographed. Eight males and eight females played the violin, five males and one female played the viola, and one male played both instruments.

Past skin disease

Nine of 14 males (64%) and two of nine females (22%) gave a history of acne. Three of the males had acne at the time of our examination. Two males gave a past history of eczema.

Age of onset of 'fiddler's neck'

The relationship of the age of onset of 'fiddler's neck' to the length of time that the subject had been playing seriously, and the average daily hours of practice, is shown in Table 1. The condition does not usually develop unless the subject is practising for several hours a day.

TABLE 1. Age of onset and hours of practising

Age of starting to play seriously	9-18 years (Average 14.1)
Age of onset of fiddler's neck	12-30 years (Average 17.1)
Hours of practising	1-7 hours (Average 3.9)

Clinical features

Table 2 shows the clinical features noted in 22 affected subjects who were examined and photographed. The typical site of the lesion is shown in Fig. 1.

The most characteristic finding, present in 82%, was an area of localized lichenification of the skin on the left side of the neck, just below the angle of the jaw. Mild pigmentation and/or erythema in this localized patch of skin was also frequently noted. In addition to the above findings, small localized inflammatory papules were present within the area of lichenification or erythema in 91% of cases

TABLE 2. Clinical features in 22 subjects

	%
Lichenification	82
Pigmentation	77
Area of erythema	50
Scaling	41
Cyst formation	32
Scarring	18
Inflammatory changes:	
Papules within area of fiddler's neck	91
Larger areas of induration within area of fiddler's neck	64
Pustules within area of fiddler's neck	18
'Satellite' inflammatory lesions outside area of fiddler's neck	36

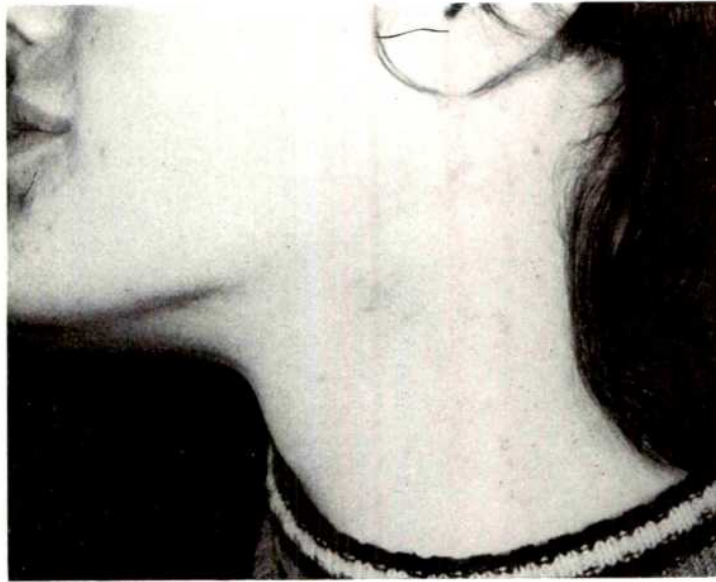


FIGURE 1. Typical site of 'fiddler's neck'.

(Fig. 2); larger areas of inflammatory induration were present in 64% and frank pustules were present in 18%. In some individuals with severe inflammatory lesions there was localized scaling. 32% of subjects had evidence of cyst formation (Fig. 3) and 18% showed some scarring. In six males and two females (36%) inflammatory papules or pustules—satellite lesions—were present in the area of skin surrounding the lichenified area. Three of these six males had active acne at the time of the examination, but the other three males and both females showed no sign of acne elsewhere.

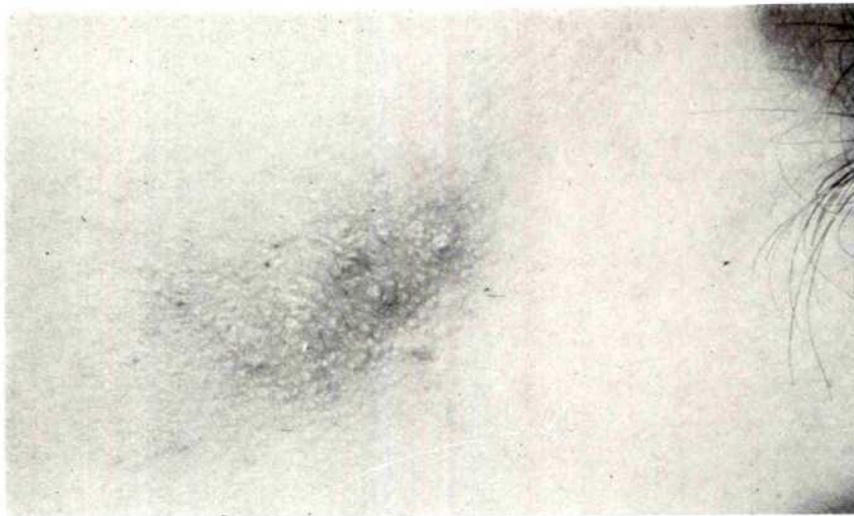


FIGURE 2. Localized area of lichenification with slight pigmentation and local inflammatory papules.

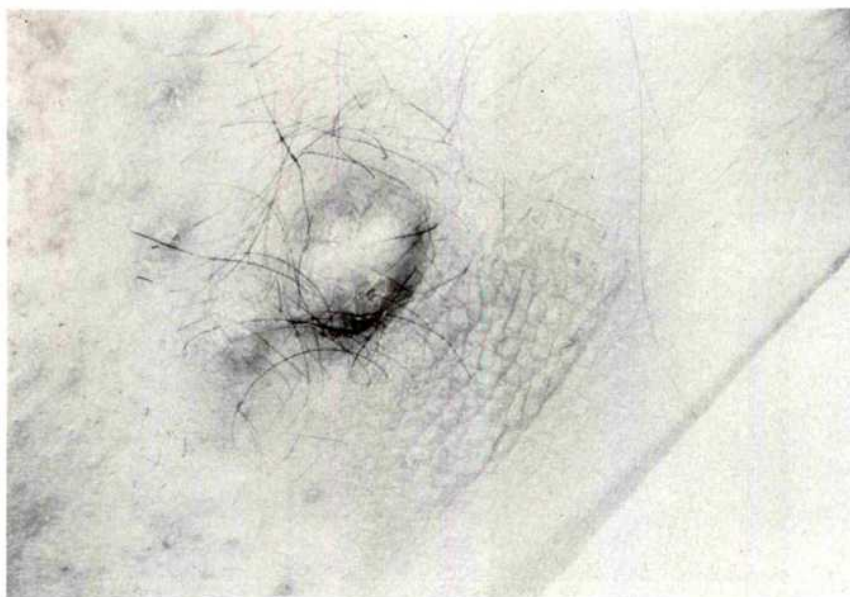


FIGURE 3. Lichenification and cyst formation.

Subjects' views on causation of 'fiddler's neck'

Table 3 shows the number of affected subjects answering the questionnaire who thought that the severity of their 'fiddler's neck' was related to such factors as their grip on the violin, weather conditions, the 'fit' or material of the chin rest, vibration or (females only) menstruation. The answers given to these questions may be criticized on the grounds that the views expressed may have been dictated, at least in part, by the beliefs of their teachers and colleagues, rather than by the subject's own experience. It is, however, clear that a high percentage thought that their grip on the violin and the 'fit' of the chin rest were the most important aetiological factors.

Histology

A skin biopsy was carried out on one subject. This showed changes of hyperkeratosis and acanthosis with evidence of follicular plugging. In the dermis there were parts of two small epidermoid cysts, one of which showed a foreign body reaction.

TABLE 3. Percentages of subjects believing that the following factors are important in the causation of fiddler's neck

	o/ o
Subject's 'grip' on the violin	91
'Fit' of the chin rest	78
Weather conditions	61
Material of the chin rest	26
Vibration	22
Menstruation (females only)	22

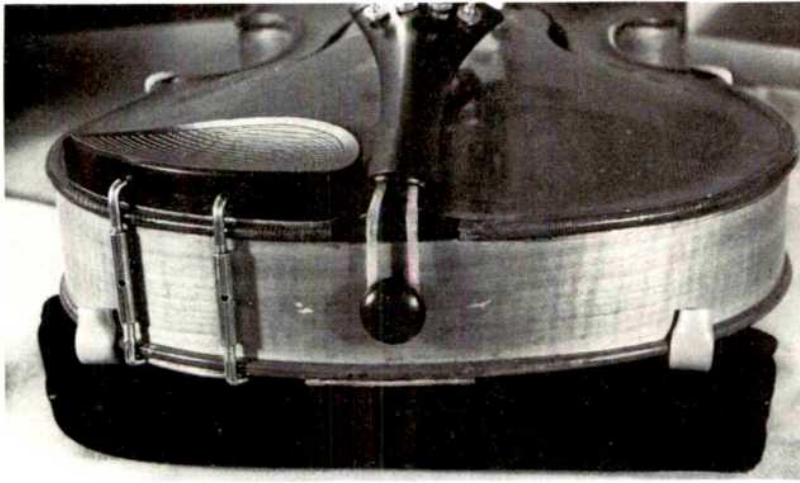


FIGURE 4. Violin showing position of chin rest. A shoulder rest is attached to the undersurface of the instrument.

DISCUSSION

The chin rest of a violin or viola is usually made of plastic or wood and is clamped to the upper surface of the violin by a metal fixing (Fig. 4). The dished and shaped upper surface is normally sited so that it is just behind the mid-line of the instrument in the playing position (side chin rest), but some violinists prefer to use a 'rest' which is actually sited in the mid-line (central chin rest). The purpose of the chin rest is to keep the player's chin off the body of the fiddle and also to give it a ridge to grip. With a suitable height and pattern of chin rest, it should be possible for the player to hold the instrument in place on the clavicle by means of pressure from the side of the chin and neck without any assistance from the left hand. The ease with which this can be attained depends partly on the length of the neck and many violinists, especially those with a long neck, add padding over the clavicle or use a shoulder rest which fits onto the undersurface of the violin in order to raise the violin off the shoulder and, thus, allow it to be held more securely by the chin.

'Fiddler's neck' occurs in the area of skin that is in closest apposition to the chin rest, and is usually sited below and perhaps slightly anterior to the angle of the mandible on the left side. The way in which the present survey was carried out did not allow us to ascertain the actual incidence of the condition, but it was clear that this was very high—virtually every member of the Strings Section of the Academy of the B.B.C. Orchestra showed some evidence of the typical skin changes. Although 'fiddler's neck' is very common among professional violinists, it is thought by many to be more troublesome in students, who are more likely to have a poor technique, and is said to be less of a problem in those who have a secure grip on the instrument with a suitable chin and shoulder rest.

From our study it seems probable that several factors, besides the duration of practice, play a part in the causation of this condition.

(1) *Pressure of the fiddle on the neck*

Increased pressure on the skin of the neck tends to occur when technique is poor or the grip of the chin on the chin rest is inadequate. Tiredness and emotional music were noted by some subjects to make the condition worse—possibly by making them more tense and thus increasing the pressure of

the fiddle on the neck. One girl noted that she was more tense when playing in a small group, where she could hear her own individual playing, than when playing in a large orchestra.

(2) *Friction between the chin rest and the skin*

This is also an important factor and is more likely to occur in those with an insecure technique. It is made worse by a poor fitting chin rest or an inadequate shoulder rest. The interposition of long hair between the chin rest and the skin may exacerbate frictional effects.

(3) *Hygiene*

Several subjects noted that hot weather, excessive sweating and poor hygiene made their skin conditions worse.

(4) *Instrument*

Viola players are believed to be more prone to develop 'fiddler's neck' than violinists because the instrument itself is larger and heavier. Our survey was not large enough to confirm or deny this.

When the histological and clinical features of the condition are taken together, there seem to be several possible mechanisms for the causation of the condition. Friction and pressure together may give rise to lichenification, pigmentation and erythema. The localized acne-like changes, so prominent in many patients, may be due in part to a combination of pressure, friction and occlusion. Mills & Kligman (1975) have described a condition which they term 'acne mechanica', in which acne changes in predisposed subjects are positioned by the effects of local pressure, friction, rubbing, squeezing or stretching, and it seems likely that similar factors may be important in 'fiddler's neck'. It is possible to speculate that some of the satellite inflammatory lesions noted in this study may occur as the result of occlusion and/or sepsis in an area of skin that is partly occluded but not subjected to major pressure and friction. Cyst formation was a feature in a number of our cases. It is known that epidermoid cyst formation is relatively common on amputation stumps (Allende, Levy & Barnes, 1963) and it is assumed in these cases that mechanical shearing forces implant fragments of epidermis in the dermis. Although epidermoid cyst formation in 'fiddler's neck' may occur as a result of the acne-like changes, it is just conceivable that persistent shearing stress on the skin may be a cause of injury to the follicular epithelium and, thus, be a factor in precipitating cyst formation. Many patients noted that their skin condition became worse in conditions of high temperature and humidity, and with excessive sweating and poor hygiene. Although no bacteriological studies were carried out, it seems likely that, at least in some cases, secondary infection was a factor in causing worsening of the condition.

ACKNOWLEDGMENTS

Our thanks are due to Mrs M.Clapham, Mr Brian Mountain and the members of the Academy of the B.B.C. Orchestra, and to Mrs F.Deller, Professor S.Griller and the students of the Royal Academy of Music.

REFERENCES

- ALLENDE, M.F., LEVY, S.W. & BARNES, G.H. (1963) Epidermoid cysts in amputees. *Acta dermato-venereologica*, **43**, 56.
 MILLS, O.H., Jr & KLIGMAN, A. (1975) Acne mechanica. *Archives of Dermatology*, **3**, 481.
 RONCHESE, F. (1948) *Occupational Marks and Other Physical Signs: a Guide to Personal Identification*. Grune & Stratton, New York.

Case Reports

Cyclophosphamide induced nail pigmentation

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SUMMARY

Cyclophosphamide therapy may occasionally cause black pigmentation of the nails. We report five cases with this side effect and review the data on eleven cases in the literature. These changes start in the proximal nail beds and progress distally; on withdrawal of cyclophosphamide, clearing of the nail pigmentation proceeds in a similar fashion. The development of the nail pigmentation does not bear any relation to the primary condition for which cyclophosphamide was prescribed. The dose of the drug before the onset of pigmentation ranged from 1.2 to 12.3 g; the duration of treatment ranged from 10 days to 26 weeks. The mechanism of the nail pigmentation is unknown.

Cyclophosphamide is an alkylating agent used commonly in the therapy of a variety of haematological and other malignancies and as an immunosuppressive agent.

Various cytopenias, haemorrhagic cystitis, alopecia, gonadal dysfunction, hyponatraemia, myocardial and pulmonary damage have been associated with the use of cyclophosphamide.

There have been occasional reports of black pigmentation of the nails associated with cyclophosphamide therapy. We have observed five instances of this interesting side effect in our patients in the haematology service at Cook County Hospital.

CASE REPORTS

Case 1

A 65-year-old black woman was admitted because of a right neck mass. Diagnosis of IgG lambda multiple myeloma and extraosseous plasmacytoma was established by immunological data, biopsy of the neck mass and bone marrow studies. Serum IgG was 2230 mg/dl, IgA 115 mg/dl and IgM 26 mg/dl. Urine was negative for Bence Jones protein. Blood urea nitrogen, serum electrolytes, serum calcium and uric acid were normal. Chest X-ray showed an anterior mediastinal mass. Radiotherapy (4200 rads in air) was given to the right side of the neck, axillary masses and the mediastinum, and therapy with cyclophosphamide and prednisone was started. The dosage of cyclophosphamide varied between 50 and 150 mg orally daily. Black discolouration of the nail beds was noted 6 weeks after

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initiation of cyclophosphamide therapy. There was no skin or mucosal pigmentation. The patient had received 3550 mg of cyclophosphamide at the time nail pigmentation was noted. Therapy was changed to high dose intermittent melphalan and prednisone, but the nail pigmentation has persisted over 27 months of follow-up.

Case 2

A 64-year-old black woman was diagnosed to have lambda light chain myeloma. Local radiotherapy was given to the right ilium to control local pain and therapy was begun with oral cyclophosphamide, 150 mg daily. Black pigmentation with longitudinal ridges of the nail beds was noted after 4 weeks of cyclophosphamide therapy to a cumulative dose of 5100 mg. Hyperpigmentation of the dorsal and palmar aspects of both hands was also noted. The feet were not affected. The 8.00 a.m. and 4.00 p.m. serum cortisol levels were studied at this time and were found to be normal. Pigmentation progressed distally to involve the entire nail and persisted during the following 4 years of cyclophosphamide treatment. Discontinuation of cyclophosphamide resulted in gradual clearing of the nail pigmentation starting proximally and progressing distally over 6 months (Figs 1 and 2).



FIGURE 1. Pigmentation involving the entire nail while on cyclophosphamide therapy.

Case 3

A 50-year-old black woman was diagnosed to have malignant lymphoma (stage IV B) and combination chemotherapy with cyclophosphamide, vincristine and prednisone was started. Black pigmentation of the nails involving the first and the second fingers of both hands was noted after 6½ months of therapy and a cumulative dose of 12.3 g of cyclophosphamide. Skin and mucosae were not affected. The patient is on maintenance chemotherapy and the nail pigmentation persists.

Case 4

A 65-year-old black man was diagnosed to have chronic lymphocytic leukaemia and treatment was initiated with cyclophosphamide, vincristine and prednisone. Pigmentation of the proximal nail



FIGURE 2. Clearing of nail pigmentation after discontinuation of cyclophosphamide.

beds of both hands was noted after 6 weeks of chemotherapy and a total dose of 4.5 g of cyclophosphamide orally. There was no pigmentation of the skin or mucosae. Nail pigmentation progressed distally and involved the entire nail over the ensuing 4 months. Cyclophosphamide was discontinued 6 months later because of haemorrhagic cystitis, and clearing of the proximal nail beds was noted 2 months later. He has since been on chlorambucil therapy and the nails have remained free of pigmentation.

Case 5

A 14-year-old black woman with metastatic liposarcoma was treated with combination chemotherapy with adriamycin, vincristine, DTIC and cyclophosphamide. She developed black nail pigmentation after 7 weeks of therapy and a total dose of 1.2 g of cyclophosphamide. Currently, she is receiving cyclophosphamide intravenously every 8 weeks. She has developed horizontal bands of black pigmentation of the nails separated by normal looking areas. Adriamycin has been discontinued after a total dose of 450 mg/m² but the nail pigmentation persists unchanged for the follow up period of 6 months.

DISCUSSION

Pigmentary abnormalities of the nails have been reported with the systemic use of drugs such as phenolphthalein (Campbell, 1931), arsenic (Mee's lines), chloroquin (Tuffanelli, Abraham & Dubois, 1963) and quinacrine (Barr, 1944). Cancer chemotherapeutic agents known to be associated with nail pigmentation include cyclophosphamide (Amar Inalsingh, 1972; Harrison & Wood, 1972; Mani, Natarajan & Jeyraj, 1973; Romankiewicz, 1974; Shah, Rao & Patel, 1975), adriamycin (Morris, Aisner & Wiernik, 1977; Pratt & Shanks, 1974; Friestman & James, 1975), and bleomycin (Shetty, 1977). Cases of nail pigmentation following radiation to the hands for dermatitis have also been reported (Sutton, 1952).

Data from previous reports of cyclophosphamide associated nail pigmentation have been sum-

marized in Table 1. The dose and duration of cyclophosphamide therapy ranged from 1.2 to 12.3 g (mean 5.4 g) and 4 to 26 weeks (mean 10 weeks) respectively in our cases. The shortest interval from the initiation of cyclophosphamide therapy to the onset of nail pigmentation was 10 days in a 3-year-old child (Harrison & Wood, 1972).

That the nail changes do not seem to be related to the underlying disease is shown by the wide variety of indications for cyclophosphamide treatment in the reported cases. Except for cyclophosphamide and adriamycin, none of the drugs mentioned in Table 1 has been associated with nail pigmentation. Since these changes were observed in a few cases where only cyclophosphamide was given and close chronological relation to the latter was demonstrable in all cases, it is reasonable to ascribe the aetiological role to the drug. Reversal of nail pigmentation on withdrawal of the drug in most cases lends further support to this postulate.

The pattern of nail pigmentation varied in our patients from diffuse to horizontal and longitudinal streaks; in one case the pigmentation was restricted to finger nails of the first and the second digits only. One patient (Case 2) had involvement of the toe nails, palmar and digital creases and the skin of the dorsum of the hands. The development of the nail pigmentation does not seem to have any prognostic value for the primary disease. The suggestion (Shahani & Blackburn, 1973) that nail pigmentation may indicate a poor prognosis is not corroborated by subsequent experience.

In our cases pigmentation was first noted at the base of the nail and progressed distally; and clearance of the pigmentation also proceeded in a similar fashion. This has also been observed by other authors (Harrison & Wood, 1972; Nixon, 1976; Romankiewicz, 1974). Patients receiving intermittent therapy developed horizontal pigmented bands separated by areas of normal nail. Clearance of the nail pigmentation after cessation of cyclophosphamide was not seen in Case 1; however, the patient has been receiving phenylalanine mustard (Melfalan) which may have been responsible for the persistent nail pigmentation.

In only five of the previous cases of cyclophosphamide induced nail pigmentation has the racial origin of the patients been reported; there were three East Indians and two Negroes. All our 5 patients were Negroes but approximately 80% of our hospital patients are Negroes so that this evidence merely raises the question whether dark skinned individuals have a propensity to develop nail pigmentation from cyclophosphamide.

The mechanism of pigmentation remains obscure. The initial involvement of the base of the nail at the onset, band pattern and the distal migration of the band with subsequent disappearance suggest a disturbance in the nail plate. It is reasonable to postulate that cyclophosphamide or one of its metabolites may affect the developing nail plate and bring about these changes. Flaxman, Sosis & Van Scott (1973), studying the hyperpigmentation induced by topical nitrogen mustard, concluded that interference with melanosome aggregation was the basis of hyperpigmentation and that this action was similar to the effect of ultraviolet radiation although longer lasting. The relation of this observation to the action of the closely related agent cyclophosphamide is unclear.

There have been no data concerning adrenal and pituitary function in the patients reported to date. The 8.00 a.m. and 4.00 p.m. serum cortisol levels were measured in one of our patients (Case 2) and were normal. Whether the pigmentary changes induced by cyclophosphamide are mediated through the action of ACTH or MSH is speculative and awaits studies of levels of these hormones after cyclophosphamide treatment.

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TABLE 1. Data on patients with nail pigmentation related to cyclophosphamide (CTX) therapy

Author(s)	Age (years)/sex	Diagnosis	Total dose of CTX/ duration of therapy	Other drugs	Reversibility	Remarks
Solidoro <i>et al.</i> (1966)	No details	Malignant lymphoma	No details	—	—	Nail pigmentation noted. No details given
Harrison <i>et al.</i> (1972)	3/M	Nephrosis; minimal change lesion	2.4 g/10 days	None	Yes	Brown line of pigment in the teeth at the junction with gums. Finger and toe nails affected
Romankiewicz (1974)	36/F	Systemic lupus; nephrotic syndrome	Two courses; 3 g/4 weeks, 6 g/8 weeks	None	Yes	Patient received two long courses of CTX therapy and had nail pigmentation after each course and complete reversal after withdrawal of CTX
Mani <i>et al.</i> (1973)	11/M	Nephrosis; minimal change	3.0 g/4 weeks	None	Yes	Diffuse nail pigmentation. No skin pigmentation
	33/M	Squamous cell Ca. of lung	5.0 g/25 days	None	—	Diffuse nail pigmentation. No skin pigmentation
	6/F	Nephrosis; minimal change	2.55 g/4 weeks	None	Yes	Diffuse nail pigmentation. No skin pigmentation
Priestman <i>et al.</i> (1975)	34/F	Metastatic breast cancer	—/3.5 months	Adriamycin	—	Grey, brown or black pigmentation. Longitudinal nail banding in all three patients
	51/F	Metastatic breast cancer	—/4.5 months	Adriamycin	—	
	56/F	Metastatic breast cancer	—/6 months	Adriamycin	—	
Amar Inal-Singh (1972)	31/F	—	1.8 g/4 weeks	None	Yes	Palmar pigmentation noted. Palms cleared in 4 weeks. Nails cleared over 6 months
Nixon (1976)	41/M	Hemangiosarcoma of right hip	Dose not specified, over 'several months'	Adriamycin, methotrexate	—	Horizontal banding of finger and toe nails
Shah <i>et al.</i> (1975)	65/F	Multiple myeloma	3.55 g/6 weeks	Prednisone	—	Pigmentation of nails of both hands. Pigmentation persisted after change to melphalan
	64/F	Multiple myeloma	5.10 g/4 weeks	None	Yes	Pigmentation of nails of hands and feet, palms, and dorsa of both hands
	50/F	Non-Hodgkin's lymphoma	12.3 g/26 weeks	Vincristine, prednisone	No	Pigmentation of nails of 1st and 2nd phalanges of both hands
	65/M	Chronic lymphatic leukaemia	4.5 g/6 weeks	Vincristine, prednisone	Yes	Pigmentation of nails of both hands
	14/F	Liposarcoma	1.2 g/7 weeks	Vincristine, adriamycin, DTIC	—	Horizontal black bands of finger nails. Pigmentation persists after discontinuation of adriamycin

REFERENCES

- AMAR INALSINGH, C.H. (1972) Melanonychia after treatment of malignant disease with radiation and cyclophosphamide. *Archives of Dermatology*, **196**, 765.
- BARR, J.F. (1944) Subungal pigmentation following prolonged Atabrine therapy. *US Naval Medical Bulletin*, **43**, 929.
- CAMPBELL, G.S. (1931) Peculiar pigmentation following the use of purgative containing phenolphthalein. *British Journal of Dermatology and Syphilology*, **43**, 186.
- FLAXMAN, B.A., SOSIS, A.C. & VAN SCOTT, E.J. (1973) Changes in malanosome distribution in caucasoid skin following topical application of nitrogen mustard. *Journal of Investigative Dermatology*, **60**, 321.
- HARRISON, B.M. & WOOD, C.B.S. (1972) Cyclophosphamide and pigmentation. *British Medical Journal*, **ii**, 352.
- MANI, M.K., NATARAJAN, K. & JEYRAJ, S.D. (1973) Pigmented nails—an uncommon side effect of cyclophosphamide therapy. *Journal of the Association of Physicians of India*, **21**, 799.
- MORRIS, D., AISNER, J. & WIERNIK, P.H. (1977) Horizontal pigmented banding of the nails in association with adriamycin chemotherapy. *Cancer Treatment Reports*, **61**, 499.
- NIXON, D.W. (1976) Alterations in nail pigment with cancer chemotherapy. *Archives of Internal Medicine*, **136**, 1117.
- PRATT, C.B. & SHANKS, E.C. (1974) Hyperpigmentation of nails from Doxorubicin. *Journal of the American Medical Association*, **228**, 460.
- PRIESTMAN, T.J. & JAMES, K.W. (1975) Adriamycin and longitudinal pigmented banding of fingernails. *Lancet*, **i**, 1337.
- ROMANKIEWICZ, J.A. (1974) Cyclophosphamide and pigmentation. *American Journal of Hospital Pharmacy*, **31**, 1074.
- SHAH, P.C., RAO, K.R.P. & PATEL, A.R. (1975) Cyclophosphamide induced nail pigmentation. *Lancet*, **ii**, 548.
- SHAHANI, R.T. & BLACKBURN, E.K. (1973) Nail anomalies in Hodgkin's disease. *British Journal of Dermatology*, **89**, 457.
- SHETTY, M.R. (1977) Case of pigmented banding of the nail caused by Bleomycin. *Cancer Treatment Reports*, **61**, 501.
- SOLIDORO, A. & RICARDO, S. (1966) Effects of cyclophosphamide on 127 patients with malignant lymphoma. *Cancer Chemotherapy Reports*, **50**, 265.
- SUTTON, R.L. (1952) Transverse band pigmentation of finger nails after X-ray therapy. *Journal of the American Medical Association*, **150**, 210.
- TUFFANELLI, D., ABRAHAM, R.K. & DUBOIS, E.Z. (1963) Pigmentation from antimalarial therapy. *Archives of Dermatology*, **88**, 419.

Subcorneal pustular dermatosis (Sneddon–Wilkinson disease)—therapeutic problems

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SUMMARY

A patient with subcorneal pustular dermatosis with a fatal outcome is presented. Treatment with corticosteroids, vitamin E, dapsone, sulphapyridine and levamisole was ineffective. Only systemic treatment with retinoic acid and a new aromatic retinoic acid derivative (Ro 10-9359) produced a satisfactory clinical response, but a complete remission was not obtained.

Subcorneal pustular dermatosis, first described by Sneddon & Wilkinson (1956), is a chronic, benign, relapsing pustular eruption with an unknown aetiology and pathogenesis.

The treatment of choice is diaminodiphenylsulphone (dapsone) or sulphapyridine; corticosteroids are less effective (Wolff, 1966). Successful results of oral treatment with vitamin E (Ayres & Mihan, 1974; Johnson & Cripps, 1974) and a retinoic acid derivative (Ro 10-9359) (Kaufmann, 1975) have also been reported. Moreover Schnitzler *et al.* (1977) used alternately retinoic acid and corticosteroid locally with success.

In this paper we report our findings in treating a patient with Sneddon–Wilkinson disease, including treatment with a new aromatic retinoic acid derivative (Ro 10-9359) with a better therapeutic index than retinoic acid (Schimpf, 1976), and with levamisole.

CASE HISTORY

A 71-year-old Caucasian man (body weight 44.5 kg) was seen in our department with a 10-month history of a widespread eruption on trunk and limbs, palms and soles included. The eruption consisted of numerous pustules surrounded by an erythematous areola, ruptured and dried pustules and yellow crusts. Pustules and crusts were arranged in circinate and gyrate patterns, extending at the periphery and leaving a red-brown discolouration on healing. The patient had also suffered from rheumatoid arthritis for 11 years.

INVESTIGATIONS

ESR 129 mm/h, haemoglobin 6.5 mmol/l, haematocrit 34%; WBC and differential leukocyte count were normal. Serum iron and total iron binding capacity were decreased (5 and 29 μ mol respectively).

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The levels of serum vitamin B₁₂ and folic acid were normal. Serum electrophoresis revealed a normal total protein value of 65 g/l with a hypoalbuminaemia of 26.5 g/l, a slightly elevated beta-globulin level of 14.2 g/l (normal 6–10 g/l) and a moderately elevated gamma-globulin level of 29.1 g/l (normal 7–15 g/l). Immunoelectrophoresis: IgA, IgM and IgG moderately elevated; no paraproteins could be demonstrated. Serum electrolytes, inorganic phosphate, zinc, liver and renal function tests were normal. Serological tests: rheumatoid serology (Rose-Waaler and latex fixation test) positive, anti-perinuclear antibodies dubious, LE-serology (LE cell test, ANA) and syphilis serology negative. X-ray of chest normal and of joints compatible with rheumatoid arthritis. Bacterial cultures of swabs from pustules were negative.

Immunological tests: normal phagocytosis by polymorphonuclear cells (measured by NBT-reduction); E and EAC rosettes were within normal limits.

Histology

Biopsy of a pustule showed a subcorneal cavity with numerous polymorphonuclear leukocytes, which were also scattered throughout the Malpighian layer below. The upper dermis showed a perivascular mononuclear infiltrate.

Immunofluorescence investigations

IF-studies showed no deposition of immunoglobulins or immune complexes in normal or diseased skin and no circulating antiepithelial antibodies in the blood.

TREATMENT

The response to our therapeutic approaches was judged by daily rupturing and counting fresh pustules. On admission the prednisone (up to 30 mg every other day) and dapsone (up to 100 mg daily) he had been taking for several months without obvious clinical response were discontinued. Oral treatment with vitamin E up to 1600 mg daily, and a combination of vitamin E (1600 mg daily) with prednisone (15 mg every other day) and dapsone (50 mg daily), had no effect. Vitamin E medication was stopped. Hereafter the dapsone dose was increased to 250 mg, also without any obvious effect. The serum dapsone levels (Higgins & Taylor, 1973) and urinary dapsone/creatinine ratios (Huikeshoven *et al.*, 1976) showed that dapsone absorption was normal, so the dapsone was stopped.

The prednisone dose was diminished very slowly to prevent an exacerbation of the rheumatoid arthritis.

On account of a report by Kaufmann (1975) oral treatment with retinoic acid (70 mg daily) was started and within a few days an almost complete remission was achieved for about 4 months. Because of an increasing number of pustules, and side effects to be expected from long term treatment with retinoic acid, we tried a new aromatic retinoic acid derivative Ro 10-9359 (75 mg daily); we obtained the same favourable clinical response within a few days. Unfortunately a new relapse occurred after 3 months. Therefore the Ro 10-9359 was stopped and sulphapyridine (1500 mg daily) given, however, without any improvement.

Finally we made an attempt with levamisole (350 mg twice weekly), reported to improve rheumatoid arthritis (Huskisson *et al.*, 1976). After 1 month the patient developed sudden oedema of the face, dyspnoea and a grey discolouration of the skin, interpreted by his family doctor as an allergic reaction to levamisole, which was stopped. There was no clinical improvement of the subcorneal pustulosis or of the rheumatoid arthritis during levamisole therapy. The daily number of pustules rose and 4 months after stopping the Ro 10-9359 therapy the patient had a severe exacerbation: his whole

body was covered by innumerable tiny pustules and he became emaciated from protein loss through the pustules.

Retinoic acid therapy was given again (Ro 10-9359 was not available at that moment) and within 2 weeks there was a remarkable reduction of the pustules. His general condition deteriorated, however, and he died of pneumonia. At post-mortem examination no malignancy could be demonstrated.

The total duration of the disease had been nearly 3 years. Side effects during therapy with retinoic acid and Ro 10-9359 were a dry exfoliative dermatitis of the lips and of the vestibule of the nose, slight scaling of the unaffected skin, transient hypersalivation and development of haemorrhagic scabs on the lower legs. Serum vitamin A levels were within normal limits. Serum alkaline phosphatase levels were slightly elevated during retinoic acid and Ro 10-9359 therapy.

DISCUSSION

Although recently some authors have reported a relationship between IgA myelomatosis and subcorneal pustulosis (Cream, Grimes & Roberts, 1977; Schnitzler *et al.*, 1977; Ysmail Dahlouk *et al.* 1975) we could not discover a paraproteinaemia in our patient. The anaemia, the low serum iron level, the elevated levels of gammaglobulin, IgG, IgM and IgA, and the high ESR were attributable to the rheumatoid arthritis, the hypoalbuminaemia to the rheumatoid arthritis and to the protein loss through the pustules. We do not know if there was any relationship between the rheumatoid arthritis and the skin eruption in our patient. Treatment with prednisone, vitamin E, dapsone and sulphapyridine in our patient had no obvious clinical effect. Moreover a trial with levamisole did not influence the course of the disease. Levamisole is well tolerated by most patients; skin rashes and granulocytopenia may occur mainly in patients with rheumatic diseases (Symoens & Rosenthal, 1977).

Both retinoic acid and Ro 10-9359 seemed to produce an almost immediate therapeutic effect and could be useful alternatives in the treatment of subcorneal pustulosis, although no complete remission was achieved in our patient. According to Sneddon (1977) treatment with dapsone also does not always result in complete remission.

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REFERENCES

- AYRES, S. & MIHAN, R. (1974) Subcorneal pustular dermatosis controlled by vitamin E. *Archives of Dermatology*, **109**, 914.
- CREAM, J.J., GRIMES, S.M. & ROBERTS, P.D. (1977) Subcorneal pustulosis and IgG myelomatosis. *British Medical Journal*, **i**, 550.
- HIGGINS, T.N. & TAYLOR, J.D. (1973) Colorimetric method for the quantitative determination of avlosulfon (Dapsone) in serum. *Clinical Biochemistry*, **6**, 295.
- HUIKESHOVEN, H.C.J., HONHOFF, C., VAN EYS, G.J.J.M., ANTEN, J.G.F., MAYER, J.M.A. & VAN HELDEN, H.P.T. (1976) Weekly self medication of leprosy patients monitored by DDS/Creatinine ratios in urines. *Leprosy Review*, **47**, 201.
- HUSKISSON, E.C., SCOTT, J., BALME, H.W., DIEPPE, P.A., TRAPNELL, J. & WILLORGHBY, D.A. (1976) Immunostimulant therapy with levamisole for rheumatoid arthritis. *Lancet*, **i**, 393.
- JOHNSON, S.A.M. & CRIPPS, D.J. (1974) Subcorneal pustular dermatosis in children. *Archives of Dermatology*, **109**, 73.

- KAUFMANN, J. (1975) Subcorneale Pustulosis Sneddon-Wilkinson. *Dermatologica*, **150**, 238.
- SCHIMPF, A. (1976) Zur systemischen Anwendung eines aromatischen Vitamin-A-Säure-Derivates (Ro 10-9359) bei Psoriasis und Keratosen. *Zeitschrift für Hautkrankheiten*, **51**, 265.
- SCHNITZLER, L., VERRET, J.L., SCHUBERT, B., POUPLARD, A. & SIMON, L. (1977) Pustulose sous-cornée de Sneddon-Wilkinson avec acantholyse et myélome à IgA (Étude évolutive sur 13 ans). *Annales de Dermatologie et de Vénérologie*, **104**, 170.
- SNEDDON, I.B. (1977) Subcorneale pustulöse Dermatoze. *Hautarzt*, **28**, 63.
- SNEDDON, I.B. & WILKINSON, D.S. (1956) Subcorneal pustular dermatosis. *British Journal of Dermatology*, **68**, 385.
- SYMOENS, J. & ROSENTHAL, M. (1977) Levamisole in the modulation of the immune response: the current experimental and clinical state. A review. *Journal of the Reticuloendothelial Society*, **21**, 175.
- WOLFF, K. (1966) Ein Beitrag zur Nosologie der Subcornealen pustulösen Dermatoze (Sneddon-Wilkinson). *Archiv für Klinische und Experimentelle Dermatologie*, **224**, 248.
- YSMAIL DAHLOUK, M., HAMRA KROUHA, M.S., COLONNA, P. & MARILL, F.G. (1975) Association de pustulose sous-cornée de Sneddon-Wilkinson et de myélomatoze. *Bulletin de la Société Française de Dermatologie et de Syphiligraphie*, **82**, 273.

Multiple naevocellular naevi in brothers with albinism

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SUMMARY

Multiple naevocellular naevi occurring in two albino brothers are described. The light and electron microscopic findings of the naevi are described and the nature of the pigment discussed.

Naevocellular naevi are very common and are the subject of many reports. However, naevocellular naevi in the albino are rare and only 4 cases have been reported (Ito, Noguchi & Komatsu, 1956; Hall, Eusebi & Dangelo, 1976). The cases reported here are of albino brothers with multiple naevocellular naevi. To our knowledge this is the first report of this condition in an albino family.

This paper describes the light and electron microscopic findings of biopsy specimens from the patients.

Case 1

The patient was a 9-year-old Japanese male in whom albinism had been diagnosed at birth. Both parents showed normal pigmentation, but the patient's brother was an albino (Case 2). When 7 years old, he developed a few pinkish-yellow areas on his back, which gradually increased in size. Over the next 2 years more of these areas appeared and at the time of presentation many were present on his face, trunk and limbs. He had dull white skin and yellowish white hair. The irises were pale brown and tolerance for light was poor. Ophthalmological examination revealed horizontal nystagmus and myopic astigmatism. Routine haematological and biochemical tests were within normal limits. On the face, neck, trunk and limbs, round or oval, flat or slightly elevated, pinkish yellow nodules measuring 1–7 mm in size were observed (Fig. 1). There were 56 in all and their distribution is shown in the upper half of Fig. 2. In addition to these, several miliary brown macules were seen with a predilection for areas of sun exposure.

Case 2

The patient was a 7-year-old Japanese albino male, a brother of Case 1. When 5 years old, he noticed a few pinkish-yellow areas on his chest. They gradually increased in size and in number over the next 2 years and many such lesions were present on his trunk and limbs at the time of presentation. He had dull white skin and white hair. The irises were bluish brown and tolerance for light was poor.

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FIGURE 1. Some lesions on the left side of the neck of Case 1.

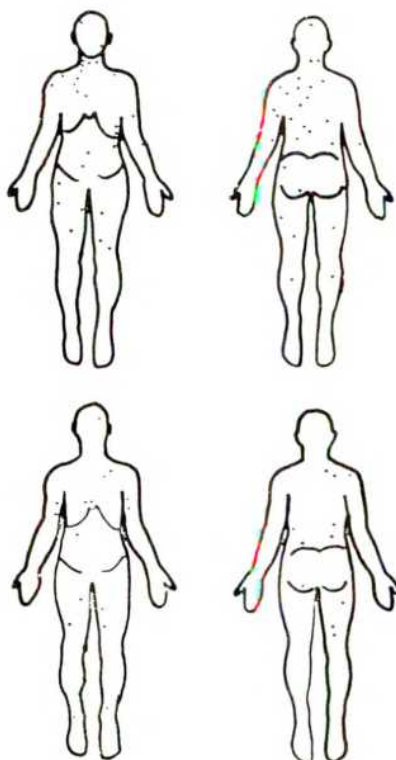


FIGURE 2. Schematic representation of the distribution of the lesions in Case 1 (upper half) and Case 2 (lower half).

Ophthalmological examination revealed horizontal nystagmus, myopic astigmatism and strabismus (left eye). Routine haematological and biochemical tests were within normal limits. On the trunk and limbs, round or oval, flat or slightly elevated, pinkish-yellow nodules measuring 1–6 mm in size were observed. There were 27 such lesions and their distribution is shown in the lower half of Fig. 2. In addition to these, there were several scattered macular lesions with a brown pigmentation situated in areas of sun exposure.

MATERIALS AND METHODS

Twenty-six and eleven specimens were taken from pinkish-yellow lesions of Cases 1 and 2 respectively. They were all removed for therapeutic reasons. One specimen was taken from uninvolved skin of each patient. Most of the specimens were used for preparation of routine sections and frozen sections. Routine sections were stained with Haematoxylin-Eosin and frozen sections were exposed to DOPA. Some specimens were examined by electron microscopy. They were pre-fixed at 4°C for 1 h with 2% glutaraldehyde buffered with 0.2 M phosphate (pH 7.4). After thorough rinsing they were immersed in 1% osmium tetroxide buffered with 0.2 M phosphate (pH 7.4) for 1 h, dehydrated in graded ethyl alcohol dilutions and embedded in Epon 812. Specimens were cut with a Porter-Blum MT-2 ultramicrotome. Ultrathin sections were stained on the grid for 1 h with a saturated aqueous solution of uranyl acetate followed by immersion for 10 min in Reynold's lead citrate and examined with a Hitachi HS 9 electron microscope. In addition, some biopsy specimens of brown lesions were cut and stained with Haematoxylin-Eosin.

RESULTS

Light microscopic findings

Although there was considerable variation in different lesions, the main pathological features in both cases were present at the dermo-epidermal junction and/or in the upper dermis (Fig. 3). In these regions cuboidal or oval cells with distinctly outlined homogeneous cytoplasm and large, round or oval nuclei were seen in clusters. Little or no melanin could be seen in the cells. Sometimes multinucleated giant cells were present, mainly in the subepidermal region. There were no mitotic or atypical cells. The DOPA reaction was positive in the cells located in the lower epidermis and the upper dermis (Fig. 4). It was slightly positive in epidermal melanocytes of uninvolved skin. Thus histologically these were features of naevocellular naevi, junctional, compound or intradermal with little or no melanin in the naevus cells.

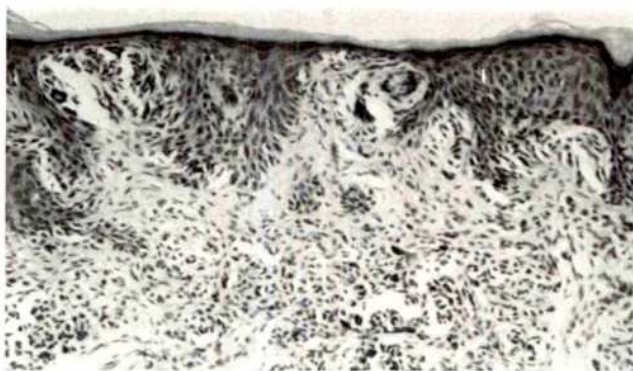


FIGURE 3. Photomicrograph of a lesion showing naevus cells mainly in the upper dermis (H & E, $\times 200$).

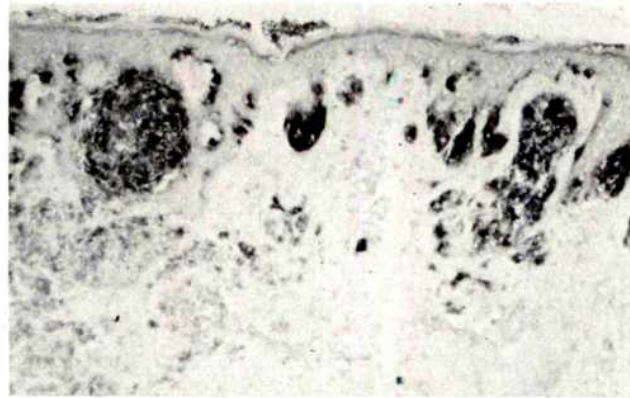


FIGURE 4. Photomicrograph showing DOPA reaction-positive cells at the dermo-epidermal junction and in the upper dermis (DOPA, $\times 200$).

Two of 26 lesions in Case 1 were junctional and 1 was intradermal. The remaining twenty-three were compound naevi. All 11 lesions from Case 2 were compound naevi. In the lesions exhibiting brown pigmentation, a large amount of melanin was present in the epidermal basal layer without a recognizable increase in melanocytes.

Electron microscopic findings

We focused on (1) epidermal melanocytes of uninvolved skin, (2) solitary naevus cells and naevus cell nests in the junctional region, and (3) subepidermal naevus cells.

Epidermal melanocytes of uninvolved skin possessed long dendrites as seen in normal human skin (Fig. 5a). However, the Golgi apparatus and rough ER were not well-developed. Most melanosomes were in stage II or III, and few were in stage IV (Fig. 5b). Some revealed the normal ellipsoidal shape ($330 \pm 40 \mu\text{m} \times 120 \pm 20 \mu\text{m}$) with striated lamellae inside, but others revealed atypical forms including, vacuole-like, abnormal spherical ($280 \pm 10 \mu\text{m}$ in diameter) and horse-shoe shapes. Solitary naevus cells in the junctional region were similar in appearance to epidermal melanocytes of uninvolved skin, although the naevus cells did not possess dendrites like those of melanocytes. They had pseudopodic cytoplasmic processes and showed relatively well-developed Golgi apparatus and rough ER. There were more melanosomes than in epidermal melanocytes and a few of those were in stage IV (Fig. 6). Naevus cells in nests in the junctional region had well-developed Golgi apparatus and rough ER. Relatively large numbers of melanosomes, mostly in stage IV, were seen. In addition, melanosome complexes were often present. The melanosomes in solitary naevus cells and naevus cells in nests revealed both shorter ellipsoidal ($310 \pm 40 \mu\text{m} \times 160 \pm 30 \mu\text{m}$) and abortive shapes. The latter were similar in appearance and size to those in epidermal melanocytes of uninvolved skin. In places there was discontinuity of the epidermal basal lamina, through which naevus cells in the epidermis communicated with those in dermis (Fig. 7). Naevus cells in the upper part of the dermis showed similar features to naevus cells in nests in the junctional region: that is, well-developed Golgi apparatus and rough ER. Most melanosomes were in stage IV. More deeply situated naevus cells showed a less developed Golgi apparatus and rough ER and fewer melanosomes (Fig. 8). The melanosomes in intradermal naevus cells were oval or round ($240 \pm 100 \mu\text{m} \times 160 \pm 60 \mu\text{m}$), some of them showing an abortive shape.

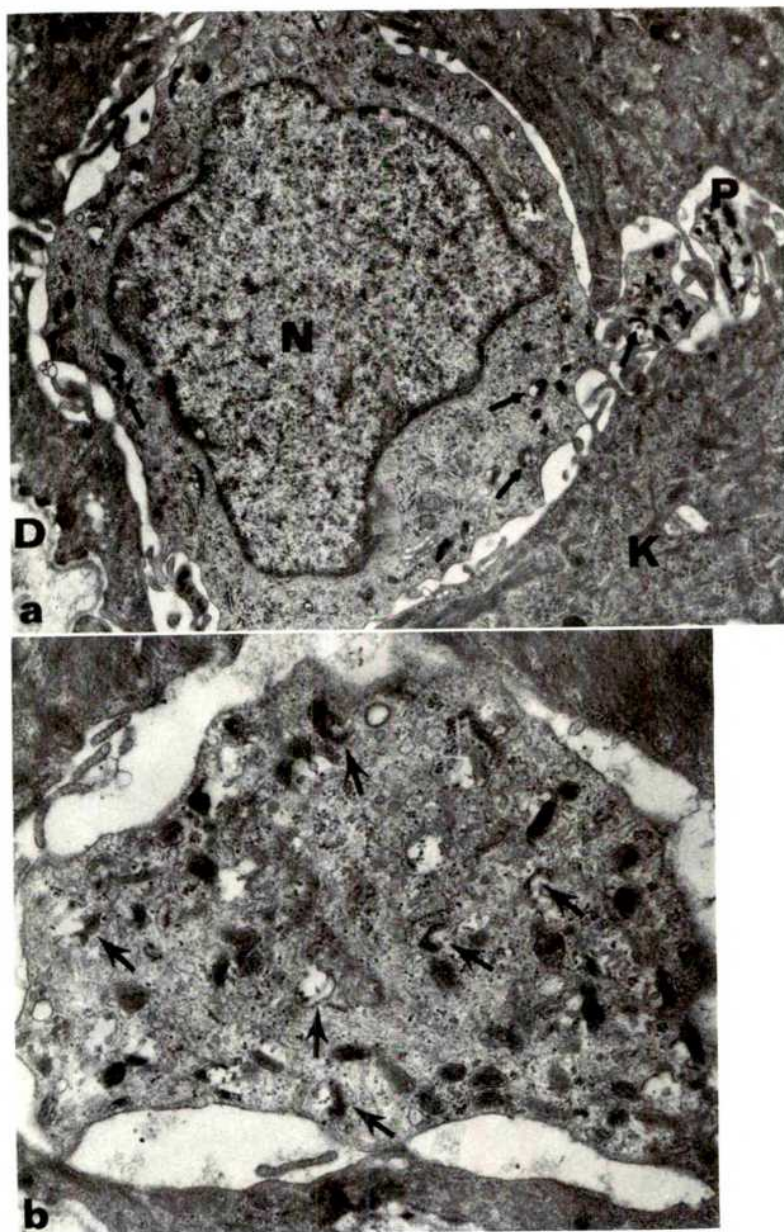


FIGURE 5. Electron micrograph of epidermal melanocytes of uninvolved skin. (a) The melanocyte possesses dendrites (P) and contains melanosomes in various stages, some of which are atypical (arrows). D, dermis; K, keratinocyte; N, nucleus ($\times 12,000$). (b) Most melanosomes are in Stage II or III, and few are in Stage IV. Some of them reveal atypical shape (arrows), that is, vacuole-like, abnormal spherical or horse-shoe shape ($\times 24,000$).

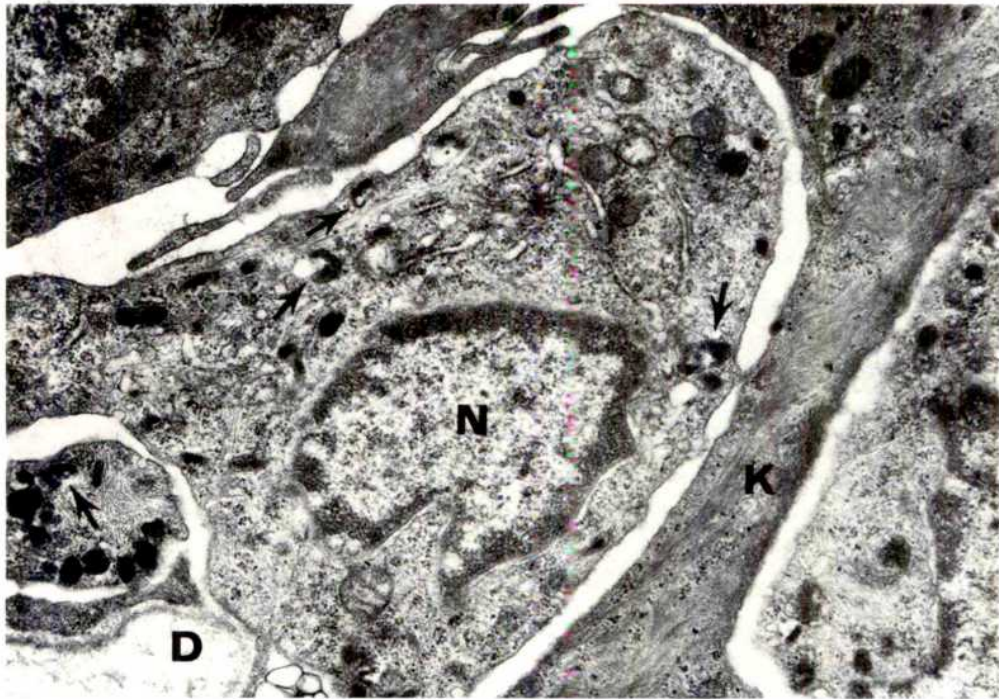


FIGURE 6. Electron micrograph of a solitary naevus cell in the junctional region. It contains several melanosomes in stage IV, as well as stage II and III, some of which (arrows) are atypical. D, dermis; K, keratinocyte; N, nucleus ($\times 24,000$).

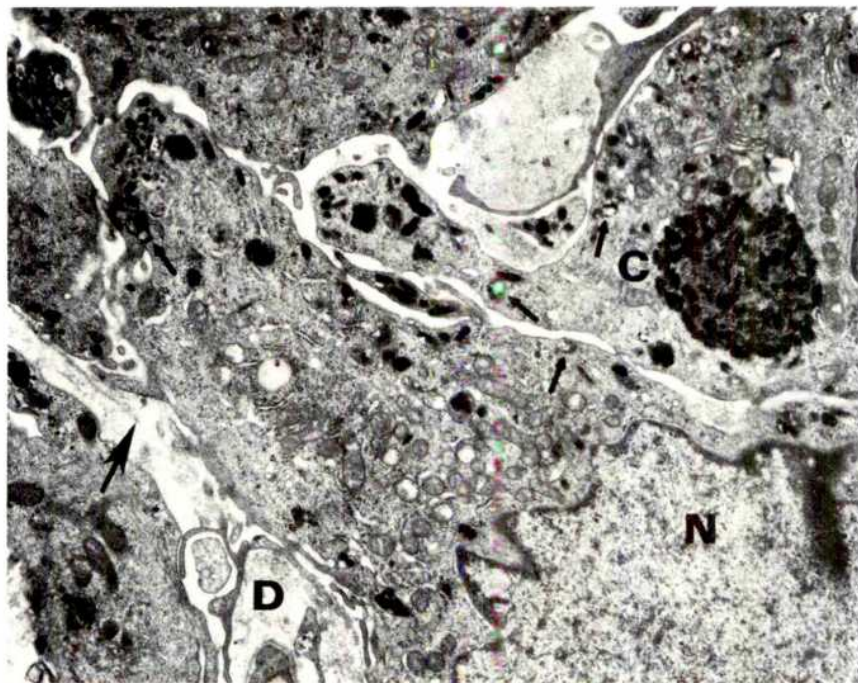


FIGURE 7. Electron micrograph of part of a naevus cell nest at the dermo-epidermal junction. A relatively large number of melanosomes, mostly in stage IV, are seen. Some of them form melanosome complexes (C). Small arrows indicate atypical melanosomes. Large arrow indicates discontinuity of basal lamina. D, dermis ($\times 12,000$).

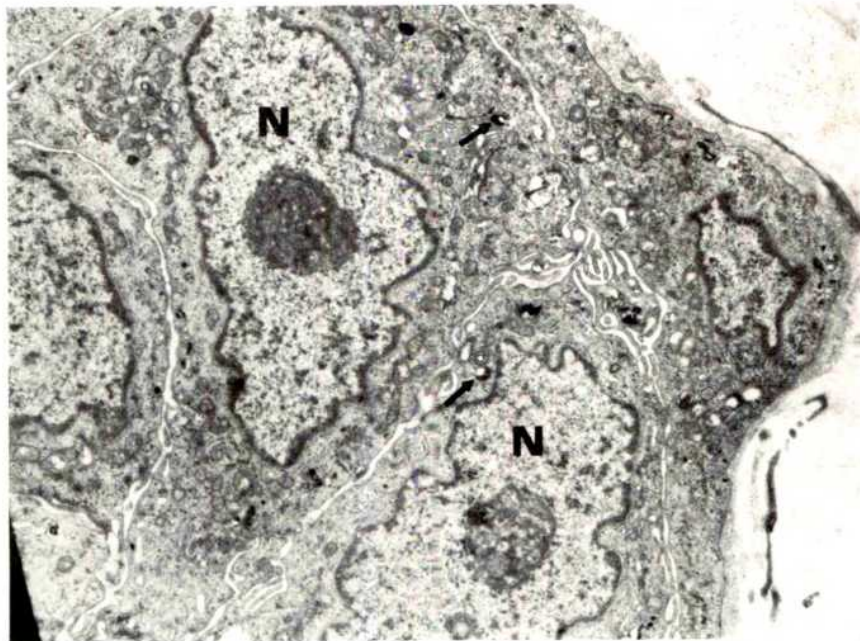


FIGURE 8. Electron micrograph of part of a naevus cell nest in the upper dermis. Only a few melanosomes, including atypical ones (arrows), can be seen in each naevus cell. N, nucleus ($\times 8400$).

DISCUSSION

The present cases are unique in that (1) the two albino brothers developed multiple moles (cellular naevi) at almost the same period, and (2) a small amount of melanin pigment was present and the DOPA reaction was positive in junctional and subepidermal naevus cells.

Ito *et al.* (1956) reported 3 albinos who presented multiple cellular naevi. Melanin pigment in the lesions was absent macroscopically and histologically, and the DOPA reaction was negative. Recently Hall *et al.* (1976) reported an albino with multiple pigmented naevi showing features of both a compound and a blue naevus. Melanin pigment in the lesion was observed macroscopically and histologically although tyrosinase activity was not measured.

Thus 3 reports, including our cases, describe different amounts of melanin pigment, ranging from complete absence to relatively large amounts, in naevi occurring in albinos. Hall *et al.* (1976) suggested that although albino melanocytes do not produce melanin, the naevus cells can overcome the genetic defect in melanogenesis and produce melanin pigment. However, we do not agree with them since melanocytes in albino epidermis may have the ability to produce melanin. Albinism can be divided into two types, tyrosinase-positive and tyrosinase-negative (Kugelman & Van Scott, 1961; Witkop, White & Nance, 1970; Jung & Anton-Lamprecht, 1971). Hishida (1973) reported that stage III and IV melanosomes were present in melanocytes of tyrosinase-positive type albinos, but not in those of the tyrosinase-negative type. The present cases also showed both a positive DOPA reaction and the presence of stage III and IV melanosomes in melanocytes in uninvolved skin. The presence of melanin pigmentation in the form of ephelides in sun exposed areas, as seen in the present cases, suggests that albino melanocytes can produce melanin pigment after sun exposure. Therefore, it is not surprising that cellular naevi occurring in tyrosinase-positive type albinos can produce melanin pigment.

In general, the ability to produce melanin is higher in junctional and subepidermal naevus cells than in naevus cells deeper in the dermis or in epidermal melanocytes in normal human skin. This is true in the naevus cells in the albino, shown in the present cases, even though the overall ability to produce melanin pigment was decreased. Thus, we think that the relative ability of melanocytes and naevus cells to produce melanin pigment is comparable in albino and in normal skin. In respect to atypical melanosomes, there are some reports that a few malformed melanosomes are present in human albino hair bulbs (Birbeck & Barnicot, 1959) and in albino mouse melanoma cells (Chian & Wilgram, 1967; Hu, Swedo & Watson, 1967). The significance of these melanosomes is not known. Perhaps their presence is related in some way to the arrested development of immature into mature melanosomes in the non-pigmented cells. The presence of atypical melanosomes in both albino melanocytes and naevus cells is interesting when one thinks of the origin of naevus cells.

Concerning the relationship between epidermal melanocytes and naevus cells, some authors (Mishima, 1965; Lupulescu *et al.*, 1973) have regarded these two types of cells as having a different genesis, whereas others (Clark & Mihm, 1969) have regarded them as identical cell types. Gottlieb, Brown & Winkelmann (1965) were able to show by electron microscopy that the fine structure of naevus cells is comparable to that of epidermal melanocytes, since not only are the melanosomes in both cells identical but also naevus cells possess pseudopodic cytoplasmic processes that, even though they are smaller, are analogous to the dendritic processes of melanocytes. Our present cases seem to provide additional information showing a close relationship between epidermal melanocytes and naevus cells.

REFERENCES

- BIRBECK, M.S.C. & BARNICOT, N.A. (1959) Electron microscope studies on pigment formation in human hair follicles. In: *Pigment Cell Biology* (Ed. by M. Gordon), p. 549. Academic Press, New York.
- CHIAN, L.T.Y. & WILGRAM, G.F. (1967) Tyrosinase inhibition: its role in suntanning and in albinism. *Science*, **155**, 198.
- CLARK, W.H., Jr & MIHM, M.C., Jr (1969) Lentigo maligna and lentigo-maligna melanoma. *American Journal of Pathology*, **55**, 39.
- GOTTLIEB, B., BROWN, A.L., Jr & WINKELMANN, R.K. (1965) Fine structure of the naevus cell. *Archives of Dermatology*, **92**, 81.
- HALL, M., EUSEBI, V. & DANGELO, I. (1976) Multiple pigmented naevi in an albino. *British Journal of Dermatology*, **95**, 199.
- HISHIDA, H. (1973) Electronmicroscopic studies of melanosomes in oculo-cutaneous albinismus. *Japanese Journal of Dermatology (Series A)*, **83**, 119.
- HU, F., SWEDO, J.L. & WATSON, H.L. (1967) Cytological variations of B-16 melanoma cells. In: *Advances in Biology of Skin Vol. VIII. The Pigmentary System* (Ed. by W. Montagna and F. Hu), p. 549. Bell and Bain Ltd., Glasgow.
- ITO, M., NOGUCHI, J. & KOMATSU, A. (1956) 'Lentigo achromiant' chez les albinos. *Annales de Dermatologie*, **83**, 631.
- JUNG, E.G. & ANTON-LAMPRECHT, I. (1971) Investigations on albinism. *archiv für Dermatologische Forschung*, **240**, 123.
- KUGELMAN, T.P. & VAN SCOTT, E.J. (1961) Tyrosinase activity in melanocytes of human albinos. *Journal of Investigative Dermatology*, **37**, 73.
- LUPULESCU, A., PINKUS, H., BIRMINGHAM, D.J., USNDEK, H.E. & POSCH, J.L. (1973) Lentigo maligna of the fingertip. *Archives of Dermatology*, **107**, 717.
- MISHIMA, Y. (1965) Macromolecular changes in pigmentary disorders. *Archives of Dermatology*, **91**, 519.
- WITKOP, C.J., Jr, WHITE, J. & NANCE, W.E. (1970) Heterogeneity in human albinism. *Journal of Investigative Dermatology*, **54**, 100.

Zosteriform lentiginous naevus with ipsilateral rigid cavus foot

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SUMMARY

A patient is presented with a rigid cavus deformity associated with a painful linear keratotic lesion on the lateral plantar border. A unilateral zosteriform lentiginous naevus is found on the same side as the foot deformity. Electrodiagnostic evaluation suggests the presence of a non-specific neuromuscular process on the affected side. This association has not previously been reported.

Very little mention is made in the dermatology literature of unilateral lentiginosis, and even less of lentiginosis with a unilateral dermatome-like distribution. The majority of the former cases are associated with central nervous system abnormalities while none of the latter are. We are presenting a patient with a unilateral zosteriform lentiginous naevus, an entity first fully described 3 years ago, with an ipsilateral rigid cavus foot and upper and lower extremity non-specific electrodiagnostic findings. Such an association has not been reported to date.

CASE REPORT

A 26-year-old Hispanic male presented to the Foot Clinics of the New York College of Podiatric Medicine with a complaint of a painful linear callus on the lateral plantar aspect and posterior heel of the left foot. It began as a discrete hyperkeratotic area lateral to the head of the fifth metatarsal and had progressed proximally until it extended the entire length of the foot, including the back of the heel (Fig. 1).

Examination revealed numerous light brown macules arranged in a zosteriform distribution confined strictly to the left side of the body. The lesions, which varied in size from 2 to 15 mm, extended from the back of the neck to the waist and were present on the posterior, lateral and anterior aspects of the trunk (Figs 2 and 3). The lesions also extended in two streaks along the volar aspect of the left arm. There was no similar involvement of the lower extremity. The lesions had been present since the first year of life, had not increased in number, and did not change appreciably with sun exposure.

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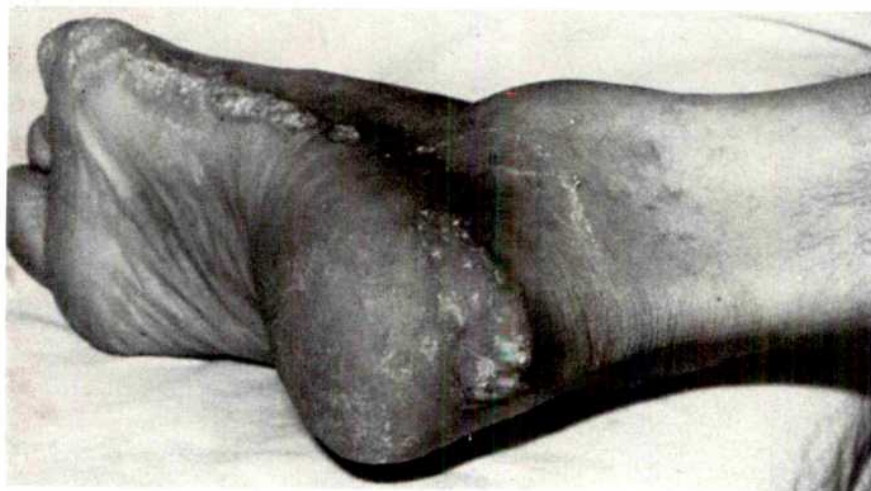


FIGURE 1. Linear keratosis on lateral plantar aspect and posterior heel of left foot.



FIGURE 2. Zosteriform lentiginous naevus following Lines of Blaschko on the anterior trunk.



FIGURE 3. Zosteriform lentiginous naevus on the back.

An additional finding of interest was a 15×12 mm hyperkeratotic plaque on the medial aspect of the thumb. This lesion, which was not subjected to abnormal friction, had been present since childhood and had grown slowly in the past few years. The left nipple and areola showed slight hypertrophy. There was no family history of any significant lesions.

The remainder of the physical examination was within normal limits except for the left lower extremity. There appeared to be a slight diffuse atrophy, with the left calf about 2 cm smaller than the right. The tibialis anterior showed a slight weakness on the left. No fasciculation was noted. The left ankle revealed a diminished range of motion and the foot had a marked rigid cavus deformity with contracted digits.

A neurological evaluation revealed normal mental status, skull examination, carotid pulses and cranial nerves. Motor examination, likewise, showed no abnormalities with the exception of a antalgic gait favouring the left side. Sensory and autonomic nervous systems were judged to be within normal limits.

Routine blood chemistry, haematology, and urine investigations were normal. Serology was non-

reactive for syphilis. Electrocardiograms showed a normal sinus rhythm. Radiological studies of the skull and chest were unremarkable. Spinal fluid was normal for glucose and protein.

An electrodiagnostic examination of the extremities revealed normal conduction velocities and evoked potentials. A borderline reduction of the left median sensory latency was found. Monopolar EMG revealed a diffuse non-specific hyperirritability of multiple muscles, more on the left than on the right, manifested by increased numbers of positive waves with occasional runs of these. The

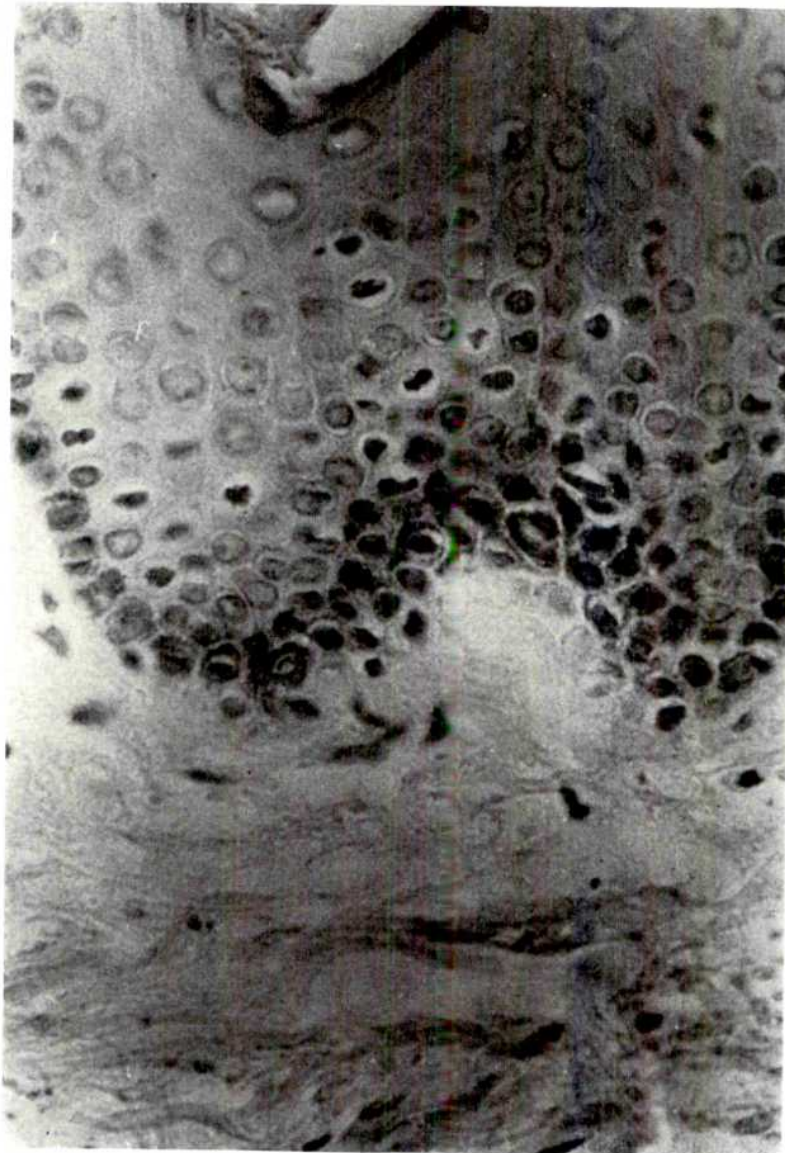


FIGURE 4. Elongated rete ridges associated with melanocytic hyperplasia and epidermal hyperpigmentation (haematoxylin-eosin, original magnification $\times 400$).

lower extremity musculature showed a greater involvement than the upper. The EMG examination was repeated once with similar results.

A muscle biopsy taken from the left gastrocnemius showed no pathological changes. A skin biopsy taken from a macule on the back revealed elongated rete ridges associated with melanocytic hyperplasia and epidermal hyperpigmentation—a description consistent with a diagnosis of lentigo (Fig. 4).

DISCUSSION

Matsudo, Reed & Homme (1973) first reported three cases of multiple lentigines which followed a unilateral dermatome-like distribution, and described the condition as zosteriform lentiginous naevus. Two of their cases had streaks of lentigines confined to the upper extremity and one in the distribution of the trigeminal nerve. Our patient has a more extensive involvement including the trunk. A close inspection suggests that the lesions follow Blaschko's lines (Jackson, 1976), a system of lines on the surface of the human body which numerous naevoid and acquired skin diseases seem to follow. These lines differ from dermatomes in that they form a 'V' shape over the upper spine, an 'S' shape on the abdomen and an inverted 'U' from the breast to the proximal arm. There is a suggestion of these configurations in our patient on his abdomen and back. Jackson reviewed theories about the origin of Blaschko's lines and discussed both embryological causes—ectodermal and/or mesodermal (mesenchymal) abnormalities—and genetic mechanisms (mosaicism) as possibilities.

There have been a small number of cases reported with multiple lentigines confined to one side. McKelway (1934) reported a case of multiple freckles in a 37-year-old woman on the left side from the hair line to the knee, with a concentration around the breast and malar regions. The patient was described as 'feeble-minded'. Cappan (1948) wrote of a mentally deficient 24-year-old male covered with freckle-like lesions involving the neck, trunk, and lower extremity in a haphazard 'peppery' distribution. The lesions were confined to the right side. The author considered the possibility of these lesions representing a forme fruste of multiple neurofibromatosis. Davis & Shaw (1964) reported a 23-year-old black female with an unusual distribution of multiple lentigines on the chest, abdomen and back on the left and chest only on the right. The macules were 4–10 mm in diameter and their long axes seemed to be directed along the dermatome lines. The lesions represented a multiple linear naevus and, unlike the above cases, no central nervous problems were involved. Davis discussed his patient in terms of a true genetic cellular mosaicism. Pickering (1973) reported a 55-year-old white female, who presented with multiple lentigines on the left trunk from above the clavicle to the level of the umbilicus. The midline in front and back provided a sharp boundary for the lesions, which had no particular pattern. The patient also suffered from focal epilepsy which was found to be associated with intracranial vascular abnormalities on the left.

Reports of generalized lentigines are somewhat more abundant. Cases have been presented with lentigines as the sole feature and others with associated abnormalities. There have been three reports of lentigo profusa—a condition with multiple lentigines over the entire body, in a haphazard arrangement, with no associated abnormalities. Gorlin, Anderson & Blaw (1969) mentioned two cases from the European literature. Rosen (1947) reported the same condition in a 20-year-old male with a profuse number of light and dark macules over the entire body with a concentration on the face and extensor surfaces of the arms. Several members of his family had a similar but less extensive involvement. Mehregan & King (1972) reported a 20-year-old white female who had an unusual collection of uniformly sized target-like pigmented naevi. Happle (1976) discussed an 11-year-old female with lesions similar to Mehregan's but which were overlapping in a cockade distribution. Zeisler & Becker (1936) presented 24-year-old white female with thousands of 1–5 mm brown-black macules over the entire body excluding the face. This patient also exhibited dermatographism.

Two well known genetic disorders, multiple neurofibromatosis (Von Recklinghausen's disease) and multiple lentiginos syndrome (Leopard syndrome), present generalized lentiginosis as part of a syndrome and are discussed by Matsudo *et al.* (1973). A number of cases involving cardiac, auditory, visual, skeletal, and sexual developmental defects in various combinations associated with multiple lentiginoses were reviewed by Gorlin (1969) in his original description of the Leopard syndrome.

Cavus deformity of the foot is usually a manifestation of some underlying neuromuscular disease (Tachdjian, 1972). A unilateral deformity is particularly suspicious and prompted our extensive neurological work-up. The previous report of zosteriform lentiginous naevus makes no mention of any concomitant neuromuscular process. The histology of the lesions in Matsudo's and our patient differ slightly. The former showed theques of junctional naevus cells at the tips of many of the papillae with increased numbers of melanocytes and increased pigment in the basal keratinocytes, while our patient's biopsy had no naevus cells but was otherwise similar. The histological picture, combined with an obviously zosteriform pattern, points to a diagnosis of lentiginous naevus. The association of a cavus deformity with abnormal electrodiagnostic findings ipsilateral with this particular skin condition has never been reported. The relationship may be due to similar developmental abnormalities of the skin and neuromuscular system or may be fortuitous.

ACKNOWLEDGMENTS

Patricia M. Schultz and Bruce C. Davis, fourth year students at the New York College of Podiatric Medicine, assisted in the preparation of this report. The neurological evaluation was done by Gerald Smallberg, M.D., Instructor in Neurology, State University of New York, Downstate Medical Center, Brooklyn, New York.

REFERENCES

- CAPPON, D. (1948) A case of unilateral lentiginoses with mental deficiency. *British Journal of Dermatology*, **60**, 371.
- DAVIS, D.G. & SHAW, M.W. (1964) An unusual human mosaic for skin pigmentation. *New England Journal of Medicine*, **270**, 1384.
- GORLIN, R.J., ANDERSON, R.C. & BLAW, M. (1969) Multiple lentiginos syndrome. *American Journal of Diseases of Children*, **117**, 652.
- HAPPLE, R. (1976) Cockade nevus: an uncommon variant of nevus cell nevus. *Hautarzt*, **25**, 594.
- JACKSON, R. (1976) The lines of Blaschko. *British Journal of Dermatology*, **95**, 350.
- MATSUDO, H., REED, W.B. & HOMME, D. (1973) Zosteriform lentiginous nevus. *Archives of Dermatology*, **107**, 902.
- McKELWAY, J.I. (1904) Lentigo: unilateral distribution, report of a case. *New York Medical Journal*, **80**, 197.
- MEHREGAN, A.H. & KING, J.R. (1972) Multiple target-like pigmented nevi. *Archives of Dermatology*, **105**, 129.
- PICKERING, J.G. (1973) Partial unilateral lentiginosis with associated developmental abnormalities. *Guys Hospital Reports*, **122**, 361.
- ROSEN, I. (1947) Generalized lentigo. *Archives of Dermatology and Syphilology*, **45**, 978.
- TACHDJIAN, M.C. (1972) *Pediatric Orthopedics*. Saunders Company, Philadelphia.
- ZEISLER, E.P. & BECKER, S.W. (1936) Generalized lentigo: its relation to systemic nonelevated nevi. *Archives of Dermatology and Syphilology*, **33**, 109.

Brief Communication

Oral therapy of pediculosis capitis with cotrimoxazole

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The efficacy of systemic administration of cotrimoxazole (CTM) in pediculosis capitis was discovered incidentally by one of us (CHS) while treating a 12-year-old girl for an upper respiratory infection. This observation led us to undertake the following study.

Twenty female inpatients aged 17–46 years with pediculosis capitis were chosen for the study. Two brands of CTM namely Septran (Burroughs Wellcome) and Ciplin (Cipla) containing 80 mg of trimethoprim (TMP) and 400 mg of sulphamethoxazole (SMX) per tablet were used.

Of the 20 patients, 12 received varying doses of CTM (CTM 2 twice daily for 3 days in 5 patients; CTM 1 twice daily for 3 days in 5 patients, and CTM $\frac{1}{2}$ twice daily for 3 days in 2 patients). Of the remaining eight patients four received TMP 160 mg twice daily for 3 days and the rest received SMX 800 mg twice daily for 3 days.

The minimal effective dose of CTM in pediculosis was found to be one tablet twice daily for 3 days. Within 8–24 h of administration of this dose the patients generally complained of intense itching of the scalp and neck, and the lice could be seen crawling in these areas. Within the next 12–48 h the lice migrated to the bed clothes and later died. During this period lice could be seen falling off the hair in a very sluggish state.

Neither TMP nor SMX showed any effect on the lice when used separately. CTM had no effect on the nits. At this stage, all the ten patients who received CTM in the minimal effective dose or above showed no evidence of lice infestation on thorough visual examination of the hair and scalp. However, the nits were still present. Therefore 8 patients were retreated with CTM 1 twice daily for 3 days, 10 days after completion of the initial CTM therapy, by which time nits had hatched. All these 8 patients were followed up for the next 15 days and were found to be completely free of nits and lice. Two patients could not be retreated as they requested an early discharge from the hospital.

COMMENT

So far all attempts to eradicate pediculus capitis infestation have been by external application only (Aviado, 1972). Many of the drugs used externally are either irritant to the eyes and skin or possess strong odours or are messy when applied and hence not well received by women for aesthetic reasons. The results of our study show that systemic (oral) administration of two courses of CTM spaced 10 days apart will eradicate the infestation without requiring any external application. The only drawback of this therapy is the intense itching of the scalp and neck experienced by the patients during the early phase of therapy. The study further shows that neither TMP nor SMX when given alone is effective but only the combination.

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CTM tablets and pure powders of SMX and TMP. We are grateful to Dr S.Chandrasekar, Professor of Medicine, Jawaharlal Institute, Pondicherry for his valuable suggestions and Dr R.Premchandran, Junior Resident in Medicine for his assistance. Help rendered by Mr N.S.Parmar, Lecturer in Pharmacology, is gratefully acknowledged.

REFERENCE

- AVIADO, D.M. (1972) Additional drugs for treatment of tropical diseases. In: *Krantz and Carr's Pharmacologic Principles of Medical Practice* (Ed. by Domingo M.Aviado), 8th edn, p. 1131. Williams and Wilkins Company, Baltimore.

PUVA-treatment of urticaria pigmentosa

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Treatment of urticaria pigmentosa (UP) has been unsatisfactory. The patients' complaints of itching, swelling and increased skin sensitivity are well known and distressing and are unrelieved by local or systemic remedies.

We have treated 10 patients suffering from UP with oral 8-methoxypsoralen (8-MOP) followed by long wave ultraviolet irradiation (UVA). The treatment schedule currently used for the treatment of psoriasis was adopted (Wolff *et al.*, 1975).

All patients showed typical generalized cutaneous involvement of late onset (except patient 10). None of them were of the telangiectasia macularis eruptiva perstans type. All diagnoses were confirmed by biopsy (except patient 10). The patients received 0.5–0.8 mg/kg 8-MOP 2 h prior to UVA irradiation (Waldmann PUVA 4000). PUVA was given 4 times a week until no wealing could be produced by mechanical irritation of the skin (Darier's sign). The data relevant to treatment are summarized in Table 1. Treatment with PUVA resulted in generalized tanning and loss of Darier's sign. While the hyperpigmented macules overlying the mast cell infiltrates showed some persistence, wealing and dermographism following physical trauma could no longer be provoked. The patients unanimously noted relief from itching and physical distress.

TABLE 1. Urticaria pigmentosa

Patients	Age/sex	Age of onset (years)	Additional diagnosis	Skin biopsy	No of treatments	Total dose (J/cm ²)	Total duration of treatment (months)	Free of symptoms after (irradiations)
K1	42 M	37	Nephrolithiasis	UP	11	32	3	9
K2	39 F	35	None	UP	9	21	1	7
K3	31 F	20	Migraine, gastritis	UP	49	417	10	14
K4	67 F	60	Cholelithiasis	UP	16	36	1	16
I5	51 F	36	Thrombocythaemia	UP	57	162	4	25
I6	48 F	38	IgG-K-paraproteinaemia, migraine	UP	28	157	2	14
I7	27 M	25	None	UP	49	307	5	27
I8	40 F	36	None	UP	25	127	1.5	16
W9	40 M	23	Migraine	UP	6	12	0.75	6
W10	7 F	1	None		8	14	1	8

*UP = urticaria pigmentosa; K = Kiel; I = Innsbruck; W = Warsaw.

In patients 5-8 an area of 10×15 cm was shielded during the entire course of treatment. Here, the cutaneous symptoms persisted unchanged. Patients 3 and 6 suffered from recurrent attacks of migraine every 2-3 weeks. In patient 3, determination of serum histamine concentration revealed an increase to $10 \mu\text{g/ml}$. Re-examination of her histamine concentration after 4 months PUVA treatment revealed normal values ($0.7-1.0 \mu\text{g/ml}$). No migraine attacks were noted. These reappeared, however, when the patient was taken off PUVA treatment for 8 weeks and also urtication of the skin could be demonstrated again. Five patients (Cases 3, 5, 6, 7, 8) were observed during post-treatment follow-up. A relapse occurred between 3 (patient 7) and 6 (patient 6) months. Also, in patient 6 this relapse coincided with the recurrence of migraine. Patient 8 remained clear for more than 6 months.

Our observations indicate that PUVA is effective in the treatment of urticaria pigmentosa. It appears to be warranted in cases in which these cutaneous abnormalities are causing severe distress. Next to psoriasis (Parrish *et al.*, 1974; Wolff *et al.*, 1975) and mycosis fungoides (Gilchrest *et al.*, 1976; Roenigk, 1977) urticaria pigmentosa can be recognized as a PUVA sensitive dermatosis. The spectrum of disorders known to be sensitive to PUVA treatment raises challenging questions as to the mode of action of this therapy.

ACKNOWLEDGMENT

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REFERENCES

- GILCHREST, B.A., PARRISH, J., TANENBAUM, L., HAYNES, H.A. & FITZPATRICK, T.B. (1976) Oral methoxsalen photochemotherapy of mycosis fungoides. *Cancer*, **38**, 683.
- PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, L. & PATHAK, M.A. (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. *New England Journal of Medicine*, **291**, 1207.
- ROENIGK, H.H. (1977) Photochemotherapy for mycosis fungoides. *Archives of Dermatology*, **113**, 1047.
- WOLFF, K., HÖNIGSMANN, H., GSCHNAIT, F. & KONRAD, K. (1975) Photochemotherapie bei Psoriasis. *Deutsche medizinische Wochenschrift*, **100**, 2471.
- WOLFF, K., FITZPATRICK, T.B., PARRISH, J.A., GSCHNAIT, F., GILCHEST, B., HÖNIGSMANN, H., PATHAK, M.A. & TANENBAUM, L. (1976) Photochemotherapy for psoriasis with orally administered methoxsalen. *Archives of Dermatology*, **112**, 943.

Comment

PUVA: A REVIEW OF ULTRAVIOLET DOSIMETRY

The treatment of psoriasis by photochemotherapy (PUVA) is expanding rapidly. The clinical results reported in the literature are encouraging with a high success rate for the clearing of the psoriatic lesions. The purpose of this paper is to compare statements of irradiance and dose made by different workers. In making this study only those publications which described the treatment of generalized psoriasis by the administration of oral 8-methoxypsoralen and UVA were considered. This meant the exclusion of papers concerned with the topical administration of trioxsalen (e.g. Fischer & Alsins, 1976) and also communications which described other applications of PUVA, e.g. treatment of vitiligo (Parrish *et al.*, 1976).

Table 1 represents a summary of UV dosimetry from those papers which contained sufficient

TABLE 1. A summary of UV dosimetry in photochemotherapy

Reference	Treatment unit	UV detector	Irradiance (mWcm ⁻²)	Typical dose per treatment (Jcm ⁻²)	Dose for clearing (Jcm ⁻²)	No. of treatments
Esther (1976)	7 'Ordinary black lights'	Not specified	0.035	(0.08)	(1.7)	20
Fry (1977)	Thorn Graph-X365 3 × 400W tubes	Not specified	5.0	2	(32)	16
Lakshmipathi <i>et al.</i> (1977)	Thorn UV and Philips Actinic Blue 05 24 × 20W and 24 × 40W tubes	Not specified	2.0	2.4	(48)	20
Melski <i>et al.</i> (1977)	GTE Sylvania	Not specified	5.6	10	247	23
Parrish <i>et al.</i> (1974)	GTE Sylvania 48 UVA bulbs	Not specified	5.6	4	(60)	15
Swanbeck <i>et al.</i> (1975)	Philips TL/08 6 × 40W tubes	Not specified	3.0	5.4	(190)	35
Walter <i>et al.</i> (1973)	General Electric F15T8- BLB	Yellow Springs Instruments Radiometer	0.82	(1)	(12)	12
Weismann <i>et al.</i> (1977)	Philips TL/08 36 × 40W tubes 12 × 20W tubes	Blak-Ray J221	4.5	3	(33)	11
Wolff <i>et al.</i> (1976)	GTE Sylvania 54 UVA bulbs	International Light Co. Photometer	6.6	5	(60)	12
Wolff <i>et al.</i> (1977)	GTE Sylvania CCT Waldmann PUVA 4000	International Light Co. Photometer	11.0	4	(50)	13

information since there are many published papers which contain no mention at all of dosimetry, e.g. Weber (1974).

There are three points to be considered about the compilation of Table 1.

(1) The aim of this paper is to present an overall look at the range of UV doses which are quoted in the literature. In order to make this as straightforward as possible, an approximate typical value of each factor was estimated where authors have given a range of values. For example, Weismann *et al.* (1977) gave patient doses per treatment in the range of $1.4\text{--}4.2 \text{ Jcm}^{-2}$ and so their mean dose was estimated to be 3 Jcm^{-2} . It is felt that in all cases where this has been done the calculated result does not distort the overall pattern.

(2) Some of the papers in Table 1 contained only two of three inter-related factors and so the third was calculated by simple arithmetic. For example Weismann *et al.* (1977) employed a mean patient dose of 3 Jcm^{-2} for each treatment and needed an average of 11 treatments for clearing of the lesions, but gave no estimate of the total dose. Consequently the total dose was calculated to be 33 Jcm^{-2} . Factors entered in the table by this method are enclosed in parentheses.

(3) There is a very wide range in the number of patients studied in each paper. The extremes are the treatment of one patient described by Esther (1976) to one thousand described by Melski *et al.* (1977). For this reason no attempt will be made to quote an overall mean value for each factor since it is also believed that there are large differences in the standard of UV dosimetry which was carried out. A study of each factor in turn yields the following general conclusions.

(1) *Treatment unit.* All radiation sources used were low pressure mercury discharge tubes with a fluorescent coating. This results in a continuous emission from approximately 320 to 400 nm with a peak at about 360 nm. This emission in the UV is generally accompanied by several lines in the visible. Since the spectral emission is probably very similar on all units, the therapeutic effectiveness of each unit per watt is likely to be equal. However this does not mean to say that currently used sources are efficiently matched to the action spectrum for the regression of lesions in psoriasis, as yet uncertain.

(2) *UV detector.* In only four out of ten cases was the detector which was used to measure the irradiance named. Of these four instances there were three different makes of UV detector. In no case were the characteristics of the detector, e.g. wavelength response, angular response, etc. discussed.

(3) *Irradiance.* Apart from the work of Esther (1976) who appears to quote an irradiance and clearing dose which is about one hundred times less than other workers, the irradiance generally quoted is around 5 mWcm^{-2} . However the detectors used to measure the irradiance have a rapid variation of response with wavelength. This suggests that the term 'irradiance' might be used rather loosely. For example, in any one paper do the authors mean:

- (a) The total irradiance measured over all the wavelengths emitted by the PUVA unit.
- (b) The irradiance between two given wavelengths.
- (c) The irradiance corrected for the 'action spectrum' of the regression of lesions in psoriasis.
- (d) Or are the authors not clear about what they are measuring?

(4) *Dose per treatment.* The above comments about the uncertainty of irradiance measurement can clearly be applied to criticisms of the dose per treatment. However even if the values quoted by Esther are ignored there is still an order of magnitude variation in this parameter.

(5) *Dose for clearing.* The values shown here give cause for concern. Although the most favoured dose is of the order of 50 Jcm^{-2} , it is disturbing to wonder how some authors can achieve clearing of lesions with only 12 Jcm^{-2} (Walter *et al.*, 1973) whilst others need to give about 200 Jcm^{-2} (Melski *et al.* 1977; Swanbeck *et al.*, 1975) to obtain, presumably, a similar biological effect. There are cases of psoriasis which can clear very rapidly for reasons that are not understood and which may partly account for this large variation in clearing dose.

Recently the Psoriasis and Photobiology Task Forces of the American Academy of Dermatology made a statement concerning PUVA therapy for psoriasis (1977). One of the considerations in this statement was the determination of the lowest effective irradiation dose to minimize potential long-term damage. The present review has clearly shown the large variation in UV doses that currently exist. It is believed that this is partly due to the uncertain approach to UV dosimetry adopted by many workers. Reliable measurement of UV radiation is not easy and some of the problems associated with dosimetry have been discussed previously (Challoner & Diffey, 1977). However until a unified approach to UV dosimetry in photochemotherapy is achieved, controlled evaluation of the benefits and risks associated with the treatment will be uncertain.

I should like to thank Mr J.R. Corfield for his encouragement in this work and Mr T.R. Harrington for his assistance with the literature search.

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REFERENCES

- CHALLONER, A.V.J. & DIFFEY, B.L. (1977) Problems associated with ultraviolet dosimetry in the photochemotherapy of psoriasis. *British Journal of Dermatology*, **97**, 643.
- ESTHER, J.R. (1976) Photochemotherapy using ordinary black light. *Archives of Dermatology*, **112**, 572.
- FISCHER, T. & ALSINS, J. (1976) Treatment of psoriasis with trioxsalen baths and dysprosium lamps. *Acta dermato-venereologica*, **56**, 383.
- FRY, L. (1977) PUVA treatment for psoriasis using metal halide quartz lamps. *British Journal of Dermatology*, **96**, 327.
- LAKSHMIPATHI, T., GOULD, P.W., MACKENZIE, L.A., JOHNSON, B.E. & FRAIN-BELL, W. (1977) Photochemotherapy in the treatment of psoriasis. *British Journal of Dermatology*, **96**, 587.
- MELSKI, J.W., TANENBAUM, L., PARRISH, J.A., FITZPATRICK, T.B. & BLEICH, H.L. (1977) Oral methoxsalen photochemotherapy for the treatment of psoriasis: a co-operative clinical trial. *Journal of Investigative Dermatology*, **68**, 328.
- PARRISH, J.A., FITZPATRICK, T.B., SHEA, C. & PATHAK, M.A. (1976) Photochemotherapy of vitiligo. *Archives of Dermatology*, **112**, 1531.
- PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, L. & PATHAK, M.A. (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. *New England Journal of Medicine*, **291**, 1207.
- SWANBECK, G., THYRESSON-HOK, M., BREDBERG, A. & LAMBERT, B. (1975) Treatment of psoriasis with oral psoralen and longwave ultraviolet light. *Acta dermato-venereologica*, **55**, 367.
- Task Forces on Psoriasis and Photobiology of the American Academy of Dermatology (1977) PUVA statement. *Archives of Dermatology*, **113**, 1195.
- WALTER, J.F. & VOORHEES, J.J. (1973) Psoriasis improved by psoralen plus black light. *Acta dermato-venereologica*, **53**, 469.
- WEBER, G. (1974) Combined 8-methoxypsoralen and black light therapy of psoriasis. *British Journal of Dermatology*, **90**, 317.

- WEISHMANN, K., HOWITZ, J. & BRO-JORGENSEN, A. (1977) Treatment of resistant psoriasis with oral 8-methoxy-psoralen and longwave ultraviolet light (PUVA). *Acta dermato-venereologica*, **57**, 73.
- WOLFF, K., FITZPATRICK, T.B., PARRISH, J.A., GSCHNAIT, F., GILCHREST, B., HONIGSMANN, H., PATHAK, M.A. & TANENBAUM, L. (1976) Photochemotherapy for psoriasis with orally administered methoxsalen. *Archives of Dermatology*, **112**, 943.
- WOLFF, K., GSCHNAIT, F., HONIGSMANN, H., KONRAD, K., PARRISH, J.A. & FITZPATRICK, T.B. (1977) Phototesting and dosimetry for photochemotherapy. *British Journal of Dermatology*, **96**, 1.

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Prevention of warfarin induced skin necrosis

SIR, In 1976 we reported the findings in a patient who developed warfarin necrosis of the skin. We briefly reviewed the literature on this condition, but were unable to shed much light on its pathogenesis. Evidence is now available which suggests that if certain predisposing events are recognized this complication of anticoagulant therapy may be averted.

The well-documented published reports indicate that at the time necrosis of the skin occurred, all patients have been over anticoagulated (van Amstel *et al.*, 1978). The prothrombin times (PT) on our patient were determined using citrated plasma and a commercial rabbit brain thromboplastin, sensitive to Factors II, VII, V, X and fibrinogen with a normal range of 12–15 s, and an ideal therapeutic range of $1\frac{1}{2}$ –2 times this value, i.e. 26–34 s.

At the time our patient developed the early signs of skin necrosis, the PT was 44 s, and 24 h later 55 s, at which time she received 20 mg vitamin K intravenously. It is now more common to start oral anticoagulant therapy with small incremental doses, such as warfarin sodium 10 mg over 2–3 days, rather than with a large loading dose, e.g. 40 mg. With large loading doses of oral anticoagulants plasma may become rapidly deficient in clotting factors, especially Factor VII (half life 6–7 h). Severe deficiency of Factor VII may not be detected in patients who are monitored by PT's using a Factor VII insensitive thromboplastin.

Other factors, such as trauma to the affected sites, often legs, buttocks, breast or genitalia, may be necessary for the development of coumarin skin necrosis, but it seems reasonable that the timely administration of vitamin K in over anticoagulated patients who develop dusky blue changes at these sites may avert this serious but rare side-effect of coumarin therapy.

REFERENCE

- VAN AMSTEL, W.J., BOEKHOUT-MUSSELT, M.J. & LOELIGER, E.A. (1978) Successful prevention of coumarin-induced haemorrhagic skin necrosis by timely administration of vitamin K. *Blut*, **36**, in press.

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Book Reviews

Psoriasis: Proceedings of the Second International Symposium. Edited by EUGENE M. FARBER and ALVIN J. COX (1977) Yorke Medical Books. Pp. 487, B/W illus.

It is not unnatural to be wary of publications which are proceedings of conferences and symposia. There are so many and the frequency of their appearance seems to be increasing. This, however, is a worthwhile book; it is better even than the first symposium proceedings organized and published by the same group in 1971. Distilled into its pages is the aggregated experience and wisdom of many respected individuals who have become well known in attempting to unravel psoriasis. There are 43 plenary papers and 54 brief communications and a special group of 9 papers on the photochemotherapy of psoriasis tacked onto the brief communications section. There are many thought-provoking sections—for example, right at the beginning in an interesting introduction Farber raises the possibility of a slow virus infection in relationship to the known association of psoriasis with certain HLA groups. I also enjoyed Van Scott and Yu's chapter on pharmacobiological leads in psoriasis but it is depressing to find that the best model for psoriasis is psoriasis itself! Their results imply that the hyperproliferative state of the epidermis is secondary to some other problem. Other very interesting work reported includes that on angiogenesis in psoriasis by John Wolf and W.R. Hubler, on an inhibitor (or inhibitors) of prostaglandin synthesis in psoriasis by Neal S. Penneys and co-workers from Miami, and on mast cells in psoriasis by Alvin Cox (what can the significance be for histamine metabolism of the increased number of mast cells in the chronic plaque and the curious target like appearance of granules on E.M.?). The reviews are good and will serve as fine starting points for research workers. Particularly well covered are the roles of anti-mitotics and antimetabolites in treatment as well as their toxic side effects. Also, and as might be expected, photochemotherapy has a good share of the action and the book includes articles from groups in Boston, Vienna and Innsbruck, Philadelphia, Munich and Stockholm. Altogether this publication can be thoroughly recommended as an important contribution. I look forward to the next symposium!

R. MARKS

Frontiers of Matrix Biology, Vol. 4: No. 1. Studies on the Biology and Pathology of Skin. No. 2. Studies in Radiobiology. Edited by L. ROBERT (1977) Basel: S. Karger. Pp. 222, 119 figures, 22 tables. Price U.S. \$54.50.

The major part of this volume is devoted to basic aspects of dermal connective tissue, and the final section discusses some biochemical effects of radiation.

The first chapter, by Borel, is a well-written review of the ontogeny and phylogeny of skin collagen. Many interesting inter-relationships are pointed out, although some references do not refer to the work discussed in the text and there are a number of minor factual errors. For example, the statement that galactose-glucose transferases are specific for one particular sequence is incorrect, since many different sequences are glycosylated in Type II collagen. Despite these minor criticisms, the chapter is highly recommended for the collagen chemist.

In the next chapter, Dhouailly reviews her work on dermo-epidermal interactions during the morphogenesis of scales, feathers and hairs. This section presents evidence that the dermis is essential for epidermal differentiation, and shows that appendage morphogenesis requires two categories of message which originate from the dermis. General readers may be somewhat daunted by the embryological jargon, but the specialist will find this a useful review of a difficult subject.

Kiraly and his co-workers then consider the ultra-structure of normal and pathological elastic fibres. Their work on solar elastosis is of particular interest, although the findings are presented in a rather uncritical manner in view of the lack of supporting data from histochemical or enzymic analysis.

The book concludes with two chapters relating to the effects of radiation on cell division. Errata

reviews our knowledge of DNA repair and replication, and assesses the role of these processes in mutagenesis and UV-induced carcinogenesis. Favre describes some recent work on the photochemistry of RNA and its relationship to the photo-regulation of bacterial growth.

This is not a book for the clinician, but cognoscenti of collagen and cell replication will find it extremely valuable. It is not cheap, however, and many workers will regret that such disparate subjects have been presented in a single volume. The editor has claimed that this conjunction will help workers studying the effects of radiation on connective tissue, but the contrived mating may well limit the sale of the book.

J.L.BURTON

Comparative Biology of Skin. Symposia of the Zoological Society of London, No. 39. Edited by R.I.C.Spearman (1977) London: Academic Press. Pp. 427. Price £14.80.

This book contains the papers contributed to a successful Symposium arranged by Dr Spearman for the Zoological Society of London in October 1976. The material falls into two parts of about equal length, designated, at least by the editor, as the 'lower' animals, i.e. invertebrates, and the 'higher' animals, i.e. vertebrates.

The first part includes papers on the integument of arthropods (S.O.Anderson), molluscs (K.Simkiss and K.M.Wilbur), platyhelminthes (Kathleen M.Lyons), nematodes (D.L.Lee), oligochaetes (K.Sylvia Richards) and pogonophores (J.D.George). Each is a detailed, up-to-date analysis of structure and function written by an expert who has made personal contributions to the field. They hold many entertaining surprises for the mammal-orientated reader. For example, in both nematodes and oligochaetes the epidermis secretes collagen to form a cuticle on its outside. Molluscan epidermis is even cleverer. It produces not only mucus and the calcareous shell, but proteins and the materials by which they are tanned into, for example, tough byssus threads for anchorage, a periostracum to coat the shell, or an elastic material, *abductin*, which forms the hinge ligaments.

Some invertebrate integuments, as Alistair Graham points out in his introduction, have even less predictable functions. It is hardly surprising that gutless tape-worms directly absorb nutrients from the intestinal contents in which they live, but there is considerable evidence that some polychaetes and echinoderms likewise absorb foodstuffs through their body walls, thus supporting a much debated theory first advanced by one Professor Pütter in 1909. The mysterious pogonophores of the deep sea, with their long worm-like bodies enclosed in tightly-fitting tubes, are unique amongst free-living Eumetazoa in having no mouth, anus or digestive tract and must thus similarly obtain nourishment through their integument.

The section on vertebrates is equally intriguing. The nature and occurrence of tubercles in fish, which function as contact organs during breeding, is reviewed by B.B.Collette. H.Fox, Mary Whitear and P.E.Budtz deal with amphibian skin, and it is left to Elizabeth Johnson to make a solitary but comprehensive contribution on mammals, sweeping across the whole field of seasonal change in the pelage and skin glands, as well as the physiological control of these functions.

The collection is completed by three papers of a different kind, sandwiched between the others. One, by R.I.C.Spearman, is a review of keratin and keratinization. The second, by P.A.Riley is—as one might expect—a succinct and erudite account of the mechanism of melanogenesis, and the third, by Rosanne Wrench, discusses the changes in mouse tail epidermis induced by tar. In one sense these papers by the editor and his close colleagues, and thus appearing by special licence, are out of place. In another sense they are the only contributions which can truly be described as 'Comparative Biology'. The overriding impression from the book is not of any pervading similarity between skins of different animals, but of the range and variety of adaptive and 'unrelatable' novelty. The concept of a 'comparative biology' may reasonably, if not always pertinently, be applied to mammalian skins; a better title for this volume might have been 'Contrasting Biology of Skin'. But it is, nevertheless, informative and often enthralling reading for all of us who consider study of the biology of the skin to be incomparable.

JOHN EBLING

La Sclerosi Sistemica Progressiva. By MARIO GIORDANO (1977) Rome: Pozzi. Pp. 150, 42 figs.

This slim monograph is an expanded version of a lecture given by Dr Giordano to the 78th Congress of the Italian Society for Internal Medicine. It presents an account of the author's 75 personal cases of systemic sclerosis, and an extensive but not comprehensive review of the literature. The Italian text is followed by an adequate English summary, occupying five pages.

In the introductory chapter we are reminded that systemic sclerosis was first described in 1753 by Carlo Curzio of Naples. Willan as early as 1796 gave Curzio the credit for this observation. Subsequent chapters consider all aspects of the disease systematically, organ by organ. Cardiac involvement was the most common cause of death in Giordano's patients; a similar finding was reported from Baltimore by Masi and Angelo, although most other published reviews show nephropathy as a more frequent cause. Whilst this book makes no fundamental contribution to our understanding of this complex disease it extends our knowledge of many of the less usual clinical features, which can create diagnostic problems, such as for example the development of cranial neuropathy within a year of the onset of Raynaud's syndrome, or the association, rare, but significant, with biliary cirrhosis. On the subject of treatment Dr Giordano is in general critical and sceptical, and thereby increases the reader's interest in the fact that he has been using griseofulvin since 1965 and claims a remarkably good response, but in certain cases only.

Those dermatologists with a special interest in systemic sclerosis will wish to read this straightforward account of wide clinical experience.

ARTHUR ROOK

Recent Advances in Clinical Immunology. Edited by R.A. THOMPSON (1977) Edinburgh: Churchill Livingstone. Pp. 299. Price £10.00.

After the boom in basic immunology a period of consolidation and expansion in clinical immunology can now be looked forward to, so that the appearance of Volume I of 'Recent Advances in Clinical Immunology' is timely and welcome. The editor of a new series is fortunate in that he is not restricted by what has gone before. Two main areas are covered in this volume—immunopathology and therapy.

Rosenthal and Franklin review the latest developments in our knowledge of amyloid, giving accounts of the chemistry, morphology and histochemistry. New information on precursors, enzymes involved in synthesis and experimental amyloidosis are lucidly dealt with, as is a revised clinical classification. Circulating immune complexes have been pursued by immunologists for several years, but no one method is capable of detecting all types of complex. Thus, the clinical immunologist has been encouraged to develop his technical ingenuity with the result that there is an almost bewildering number of methods. Zubler and Lambert provide a useful guide to the problems and techniques. The time when lymphocytes could be simply dismissed as large or small is well passed. Human T and B lymphocyte populations in the blood are described by Hayward and Greaves, who explain the tests available for distinguishing lymphocytes before giving brief accounts of the findings in immunodeficiency states and the lymphoid malignancies. A chapter on circulating phagocyte function is primarily for the laboratory worker in that it deals almost exclusively with methodology and gives little on the clinical consequences of the various disorders.

Not much has been heard lately about anti-lymphocyte globulin—an expensive biological agent whose therapeutic value remains doubtful—so a section devoted to it is largely disappointing. However, the value of replacement therapy is established and Rebecca Buckley gives a good account of indications, methods and contraindications for both humoral and cellular replacement. Morrison Smith reviews the use of corticosteroids, bronchodilators, cromoglycate and immunotherapy for allergy.

There is much here for the dermatologist whilst for those with wider tastes there are contributions on the immunology of malnutrition, schistosomiasis, aging and leukaemia.

J.J. CREAM

News and Notices

BRITISH ASSOCIATION OF DERMATOLOGISTS TRAVELLING FELLOWSHIPS

Applications are invited from Senior Registrars/Lecturers in Dermatology as well as from recently appointed Consultants and Senior Lecturers for the British Association of Dermatologists Travelling Fellowships. The Fellowships will be awarded for a period of three weeks and arrangements will be made for the Fellows to visit various centres in the United States/Canada and to attend one of the National Meetings in Dermatology. The appropriate travel and subsistence expenses of the Fellows would be borne by the B.A.D. Further information and application forms for these Fellowships can be obtained from the Hon. Secretary, Dr K.V. Sanderson, British Association of Dermatologists, 1 Wimpole Street, London W.1. The completed application forms should be returned by 16th October, 1978.

Joint Committee for Higher Medical Training and Specialist Advisory Committee in Dermatology. Correction to News and Notices. B.J.D. **97:6** p. 705

The first meeting of the SAC was held in 1971. As stated, Dr H.J. Wallace was elected Chairman, but Dr J.S. Pegum was the first Secretary, Dr A. Lyall succeeding him a year later. The other members of the SAC at its inception were: Dr H. Renwick Vickers and Professor C.D. Calnan.

Preliminary Announcement: International Symposium on Bioengineering and the Skin

An International Symposium on 'Bioengineering and the Skin' will be held at the Welsh National School of Medicine, Heath Park, Cardiff, on the 19-21 July 1979. It is intended to hold the meeting over the 3 days with four formal sessions (including poster and workshop sessions and exhibition). Progress made in understanding the physical properties of the skin and in the instrumentation for these measurements will be reviewed and original work on these themes presented.

Further details and abstract forms may be obtained from the co-organizers: Dr R. Marks, Reader & Consultant in Dermatology, Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN; or Dr P.A. Payne, Head, Bioengineering Unit, University Hospital of Wales, Heath Park, Cardiff CF4 4XW.

APPOINTMENT

Cambridgeshire Area Health Authority (Teaching)

Dr R.C.D. Staughton has been appointed Dermatologist based on Addenbrooke's Hospital, Cambridge. He qualified M.B., B.Chir., Cantab. in 1969, and M.R.C.P. (London) in 1972. His dermatology training was at Cambridge, St Thomas' Hospital and the Westminster Hospital. His special interests and publications include the systemic effects of topical corticosteroids, cutaneous reticuloses, and atopic dermatitis. At the Westminster Hospital he had special links with the Immunology Department studying cutaneous reticuloses and atopic dermatitis.

Correction

The book review which appeared in June 1977 (Vol. 96, p. 684) of *Pathology of Nails*, should have given the title of the authors as A.M. Areievich and L.T. Shetsiruli rather than A.A. Michailovich and S.L. Teofilova.

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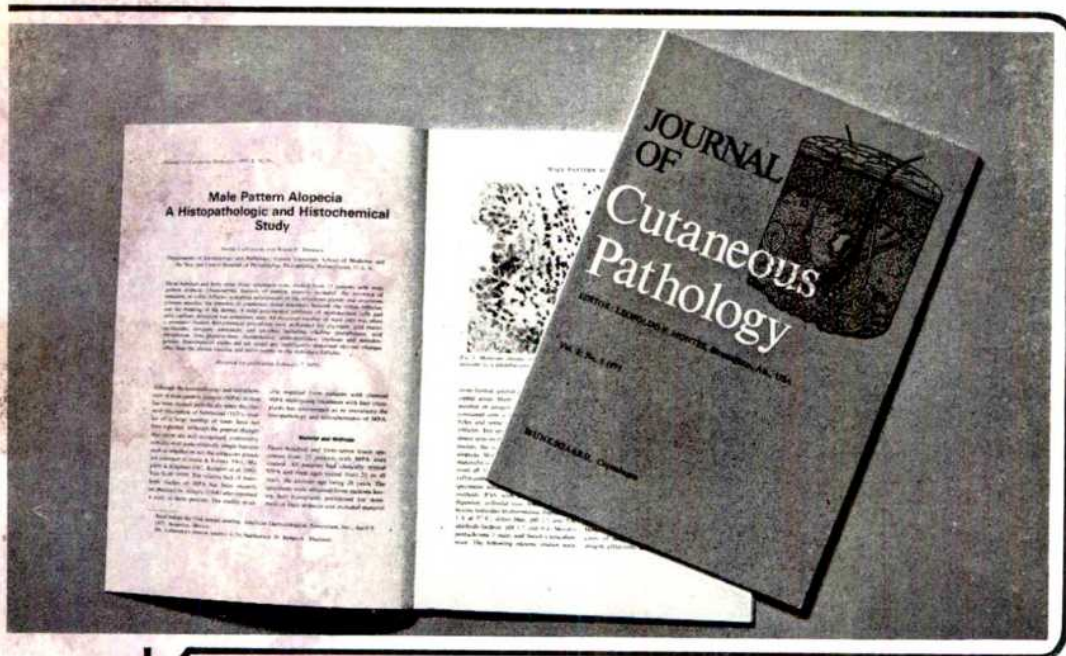
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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometer(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	¹³¹ I
hour(s)	h	millicquivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of Journal in full; (5) volume; (6) number of the first page of the article. Thus: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. Thus: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066, Blackwell Scientific Publications, Oxford.

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British Journal of Dermatology, Volume 98, Number 6, June 1978

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British Journal of Dermatology

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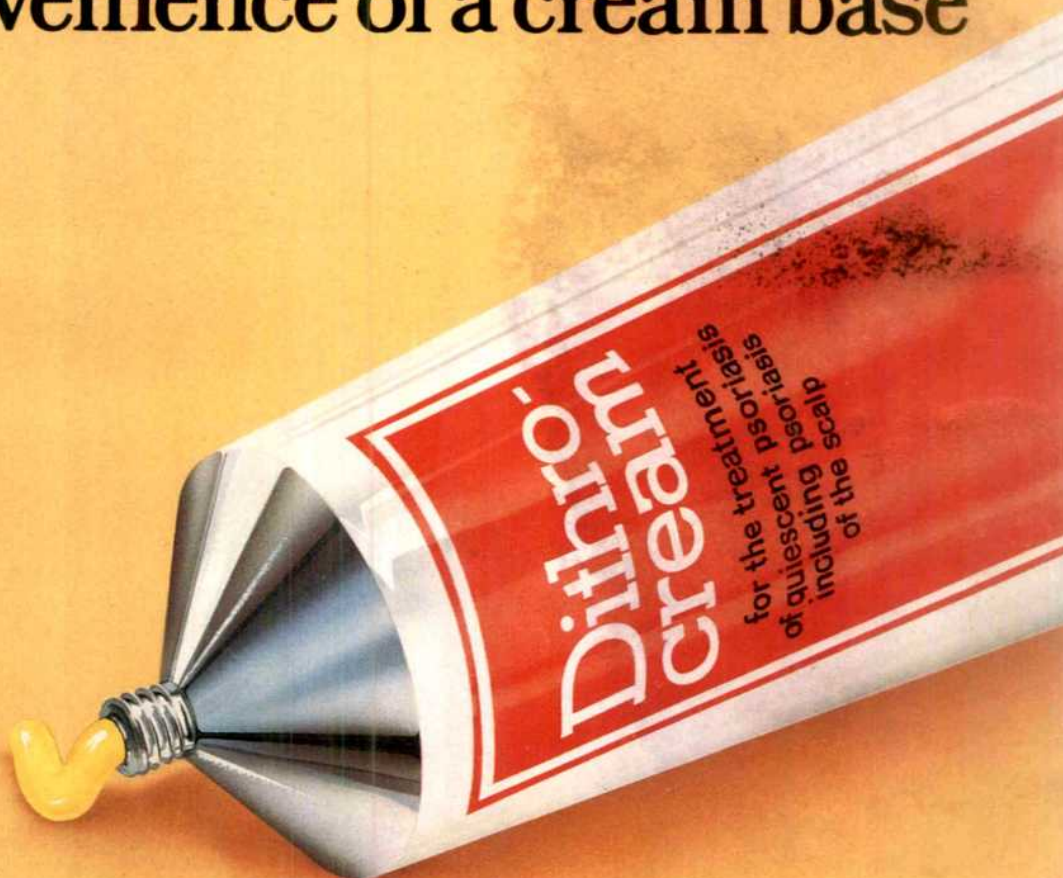
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IMPORTANT
NEW PRODUCT
FOR PSORIASIS

DITHROCREAMTM

Combines the proven efficacy of dithranol, with the convenience of a cream base



Dithrocream achieves what many pharmacists hitherto thought impossible – dithranol in a convenient vanishing cream base which is effective yet completely stable.

Being a cream formulation, Dithrocream overcomes many of the problems associated with conventional dithranol. It can be applied in small quantities and gently rubbed into the exact area of the psoriatic lesion until it is totally absorbed.

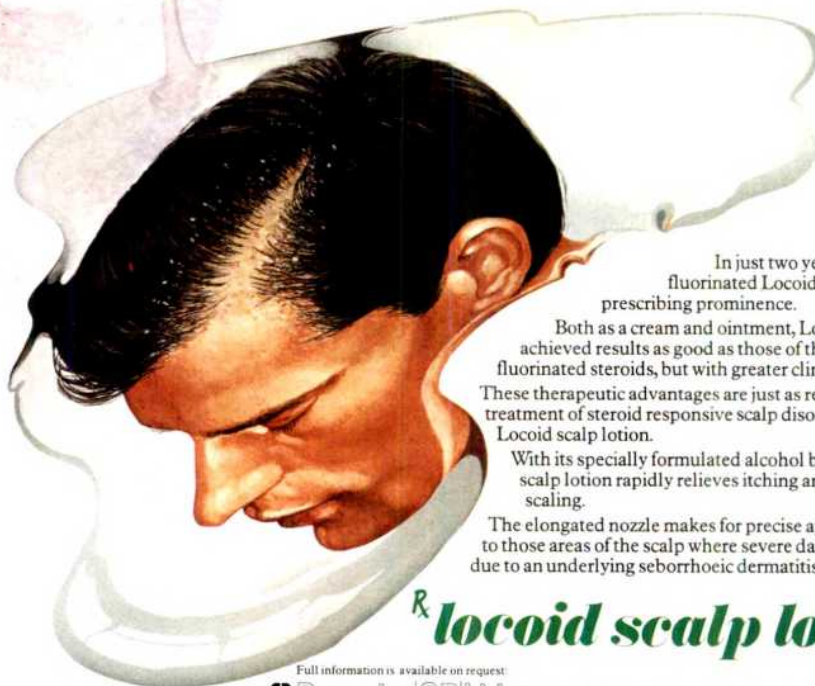
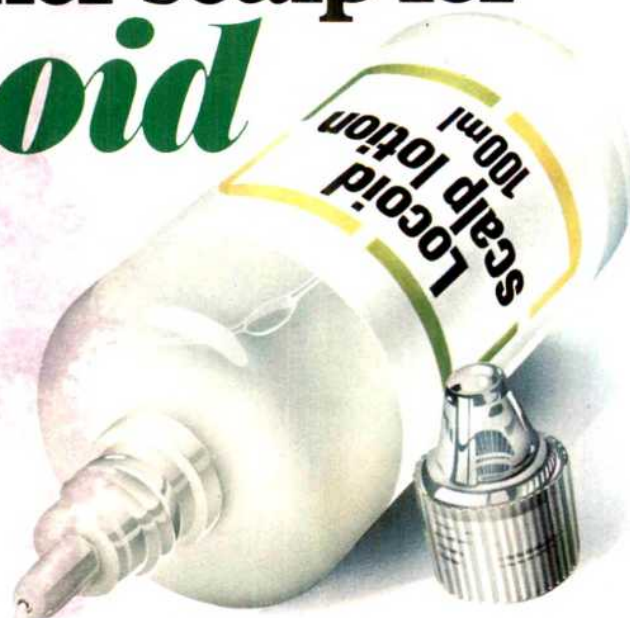
When used in this way, Dithrocream will not spread onto surrounding skin to cause burning and irritation. Staining of clothes and bedlinen is greatly reduced or entirely eliminated, obviating the need for protective dressings.

Because it is so convenient to use, Dithrocream is very suitable for most patients to apply at home.

Dithrocream: The potency of dithranol, the convenience of a cream.

DITHROCREAM CONTAINS DITHRANOL B.P. 0.25%. PL 0173 0028. DITHROCREAM IS A TRADE MARK. PATENT APPLIED FOR.
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Another scalp for *locoid*



In just two years non-fluorinated Locoid has risen in prescribing prominence.

Both as a cream and ointment, Locoid has achieved results as good as those of the potent fluorinated steroids, but with greater clinical safety. These therapeutic advantages are just as relevant in the treatment of steroid responsive scalp disorders with Locoid scalp lotion.

With its specially formulated alcohol base, Locoid scalp lotion rapidly relieves itching and reduces scaling.

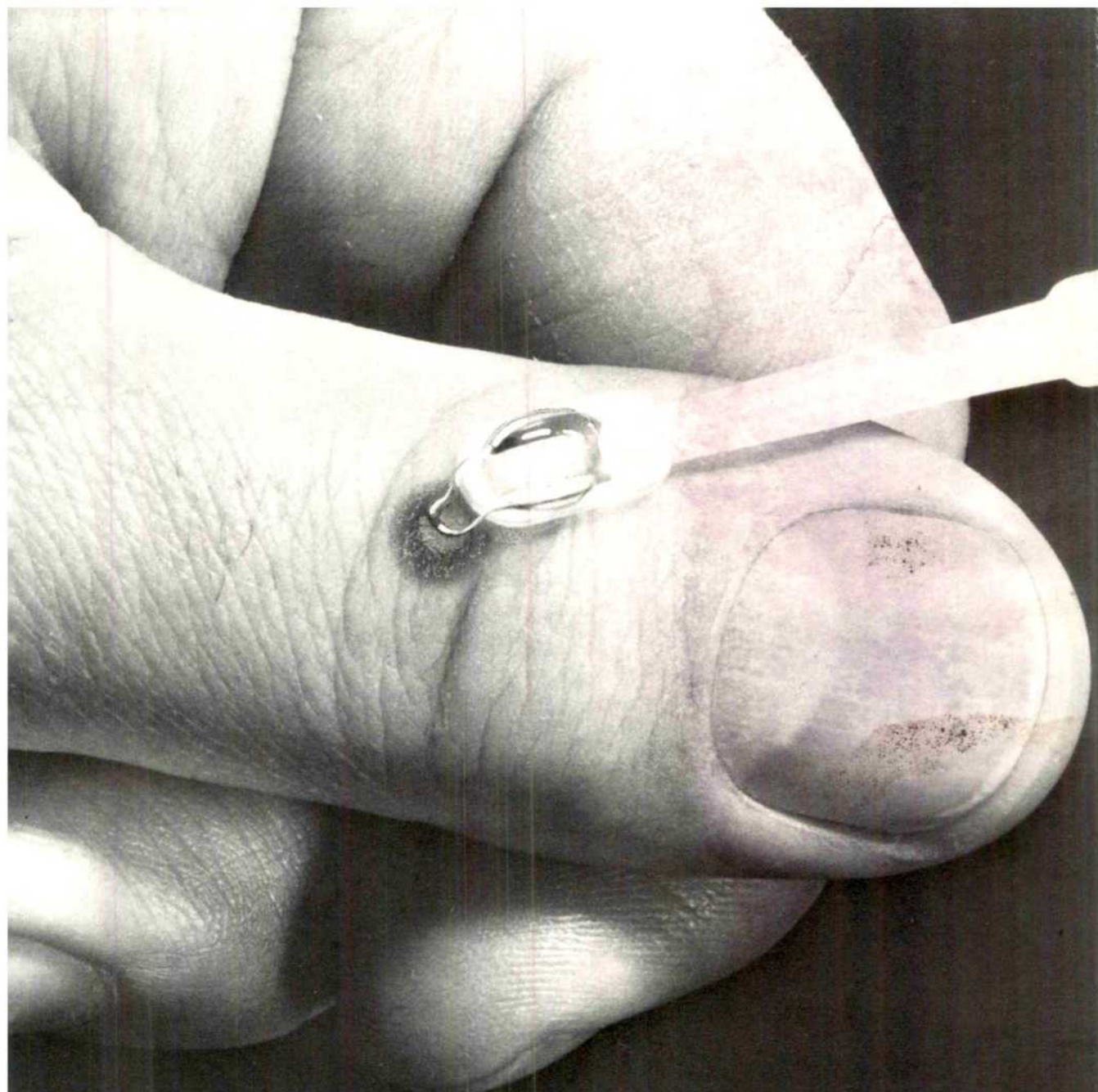
The elongated nozzle makes for precise application to those areas of the scalp where severe dandruff is due to an underlying seborrhoeic dermatitis.

locoid scalp lotion

Full information is available on request

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Locoid is the trademark for hydrocortisone 17-butyrate



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Glutarol – different because it's simple.



Glutarol contains Glutaraldehyde 10.0% w/v, and is available in 10ml bottles complete with special applicator.

Patent applied for. Full prescribing information is available.

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Glutarol
For the
treatment
of warts

10ml
Active ingredient:
Glutaraldehyde 10%
PL 0173/0022



Tri-AdcortylTM

still the best for the treatment
of infected eczemas.



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Available as cream, ointment and otic ointment. Tri-Adcortyl is a trademark of E. R. Squibb and Sons Limited.

Further information is available on request from: The Technical Services Department, E. R. Squibb & Sons Limited, Regal House, Twickenham TW1 9QT.

You can treat fungal infections

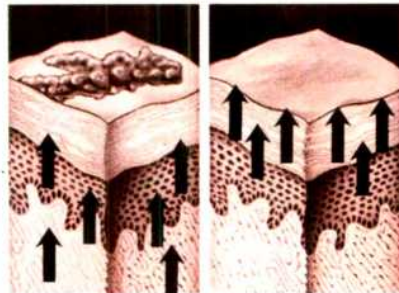


superficially. Or you can get to the root of the problem with Grisovin

Grisovin is griseofulvin, the oral antifungal antibiotic for systemic treatment of ringworm infections.

Proven in use for over 20 years, Grisovin offers all the benefits of oral over topical therapy: it is neither messy nor inconvenient to use; and for deep-seated conditions it is more effective. Most adult patients will need only one 500mg tablet twice a day. In addition, Grisovin is notable for its lack of side effects.

In the treatment of tinea infections, nail infections and scalp ringworm, the proven value of griseofulvin, its oral administration, unique action and dosage convenience, make Grisovin the effective, more acceptable therapy.



Unique action of Grisovin in skin.

1. Oral griseofulvin reaches basal layer via blood stream and protects newly formed cells from infection.
2. Outward movement of griseofulvin-protected keratin expels fungus.

Glaxo

Full prescribing information on Grisovin (trade mark) is available from:
Pharmaceuticals Division
Glaxo Laboratories Limited
Greenford, Middlesex UB6 0HE

GRISOVIN

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.

Children: usually 10mg per kg (5mg/lb) bodyweight daily in divided doses.

Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

Porphyria or severe liver disease. Griseofulvin may cause

deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions

Griseofulvin may decrease the effect of coumarin anti-coagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established.

griseofulvin

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Presentation

Tablets
125mg (bottles of 100 and 1000)
500mg (bottles of 25 and 100*)

Product Licence number

4/5060
4/5061

*Basic NHS cost (exclusive of VAT) — £7.14

new
from Glaxo
Trimovate
treats infected
dermatoses

Trimovate has been specially developed for the treatment of steroid-responsive dermatoses in the thin-skinned, moist, occluded areas of the body.

Trimovate is a topically active corticosteroid — clobetasone butyrate — is a mild steroid which offers a wide margin of safety, yet has greater efficacy than hydrocortisone. Trimovate also contains nystatin, for the effective treatment of candida infection, and a tetracycline to help insure against the possibility of bacterial invasion. Trimovate is ideal in dermatoses where candida or bacterial infection is present, suspected or likely to occur and where the use of a more potent topical corticosteroid is often undesirable.

Trimovate

Cream (clobetasone butyrate 0.05% w/w, nystatin 100,000 units per gram and oxytetracycline 3.0% w/w as calcium oxytetracycline). Available in tubes of 25 grams.
Ointment (clobetasone butyrate 0.05% w/w, nystatin 100,000 units per gram and chlortetracycline hydrochloride 3.0% w/w). Available in tubes of 25 grams.

Glaxo Leaders in topical steroid therapy

Further information on Trimovate (trade mark) is available from:
Glaxo Laboratories Ltd, Greenford, Middlesex UB6 0HE
Product licence numbers
Ointment — PL/0004/0265 Cream — PL/0004/0266

Prescribing information

Dosage and administration

Apply to the affected area up to four times a day.

Contra-indications

Viral (e.g., herpes simplex) and dermatophyte (tinea/ringworm) diseases of the skin, and tuberculous lesions.

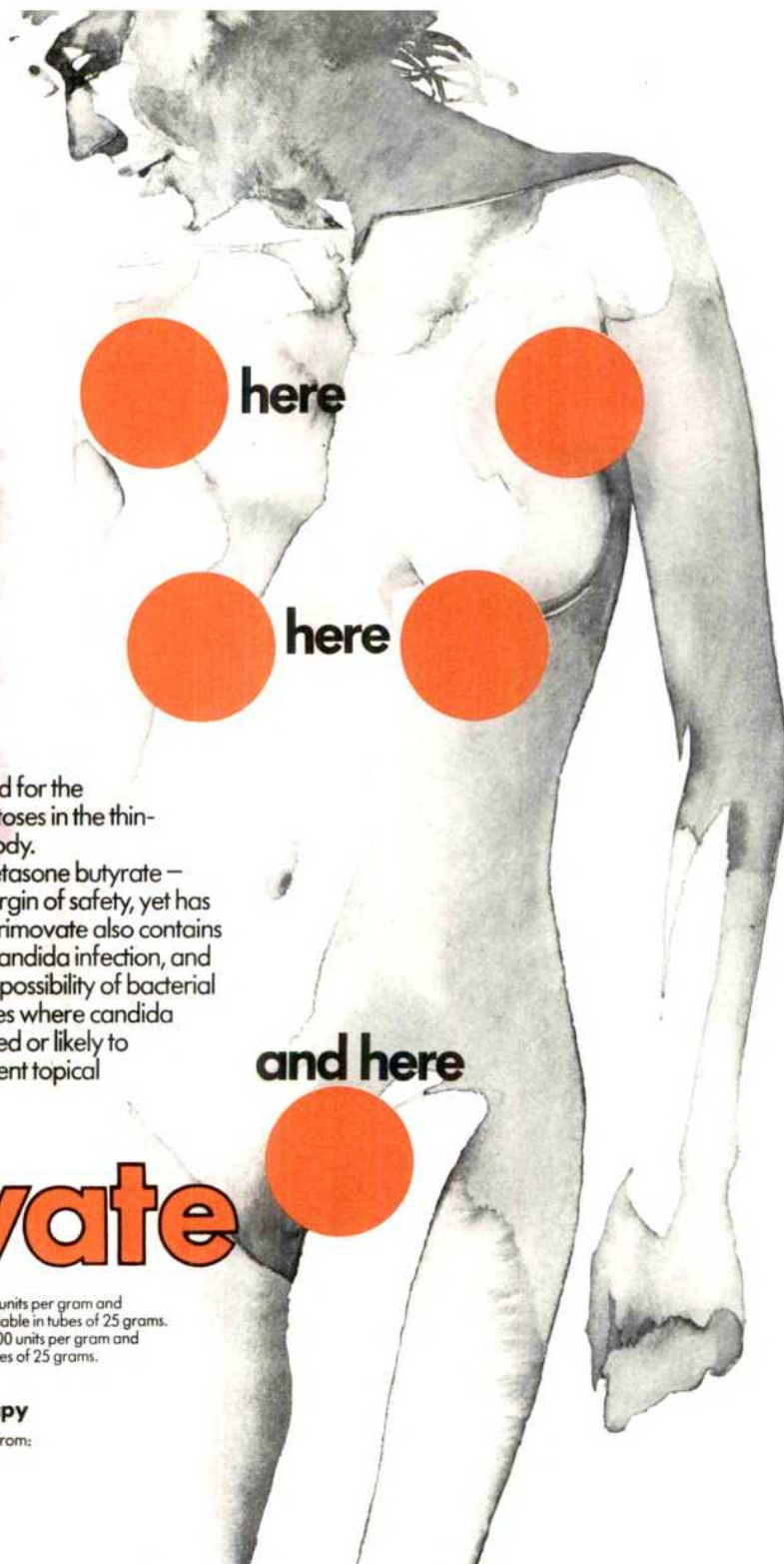
Precautions

In infants and children, long-term continuous topical steroid therapy should be avoided where possible, as adrenal suppression can occur even without occlusion. In infants, the napkin may act as an occlusive dressing and increase absorption. Any spread of

infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic chemotherapy. With all corticosteroids, prolonged application to the face is undesirable. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately. If large areas of the body were to be treated with Trimovate, it is possible that some patients would absorb sufficient steroid to cause transient adrenal depression despite the low degree of systemic activity associated with clobetasone butyrate. Local atrophic changes could possibly occur in situations where moisture increases absorption of clobetasone butyrate, but only after prolonged use.





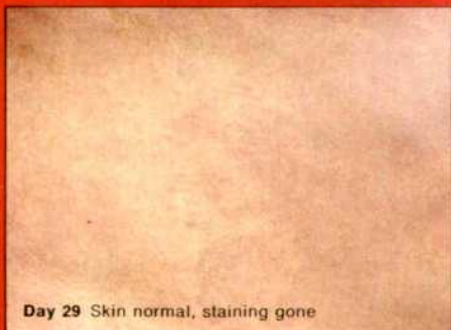
Day 1 A discrete psoriatic plaque



Day 8 After treatment with Dithrolan



Day 15 Psoriasis clear - stain remaining



Day 29 Skin normal, staining gone

Dithrolan[®]

The most effective form of dithranol

Dithrolan is the most effective formulation of dithranol in the treatment of psoriasis. Its action is so potent that, in most cases, it need only be applied two or three times a week. And the relapse rate after treatment with dithranol is far less than when steroids under occlusion are combined with dithranol.

...and easier to use

Dithrolan is much easier to apply than conventional pastes and is simple to remove. So patients can often carry out treatment at home. An instruction leaflet is enclosed with each pack.

Dithrolan-less ointment, less often, with less relapse.

Dithrolan contains 0.5% Dithranol and 0.5% Salicylic Acid.

References: 1. Brit. J. Derm 93, 205, 1975. 2. Brit. J. Derm 95, 643, 1976. 3. Practitioner 219, 833, 1977.

Full prescribing information is available.

Dermal Laboratories Limited, Talmore Place, Gosmore, Hitchin, Herts.

All you need to treat a of skin conditions. Sim



Steroid-responsive skin diseases can vary so widely, in severity, site and distribution, that a choice of activity in topical corticosteroids is needed in prescribing. That choice is available from Glaxo: three topical steroids that between them cover a wide spectrum of skin conditions – from the mildest of eczemas at one extreme to the most stubborn psoriasis at the other.

Selective use of these three steroids allows each patient to be treated with the product of lowest activity appropriate to the diagnosis and circumstances of the case. Each patient can therefore have the benefit of an optimum balance of efficacy and safety.

Wide spectrum Effectively.

PRESCRIBING INFORMATION

Dosage and administration

EUMOVATE: Apply up to four times a day until improvement occurs, when the frequency may be reduced.

BETNOVATE: Apply a small quantity two or three times daily until improvement occurs. Improvement may then be maintained by applying once a day, or less often.

DERMOVATE: Apply sparingly once or twice daily until improvement occurs. Treatment should not be continued for more than four weeks without review.

Side effects

General: With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.

Rarely, treatment of psoriasis with corticosteroids for its withdrawal is thought to have provoked the pustular form of the disease.

Precautions

Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy. With all corticosteroids, prolonged application to the face is undesirable.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

Rosacea, acne and peri-oral dermatitis, all of which can be treated with oral tetracyclines. Viral diseases of the skin, e.g., herpes simplex.

Basic NHS cost (exclusive of VAT)

Eumovate Cream or Ointment 25 gram tube £1.23
Betnovate Cream or Ointment 30 gram tube £0.96
Dermovate Cream or Ointment 25 gram tube £1.45
(also available in 100 gram tubes)

Presentation Product Licence number

Eumovate
Cream, Ointment 4/0233, 4/0254

Betnovate
Cream, Ointment 4/5121, 4/5124
Lotion 4/5130

Dermovate
Cream, Ointment 4/0219, 4/0220

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Eumovate
(0.05% clobetasone butyrate)

mild Eumovate offers greater efficacy than hydrocortisone, yet possesses a wide margin of safety.

Betnovate
(0.1% betamethasone as valerate)

medium Rapidly effective and economical, it is the preparation most frequently selected.

Dermovate
(0.05% clobetasol propionate)

strong Dermovate succeeds so swiftly, treatment need and should be of short duration only.

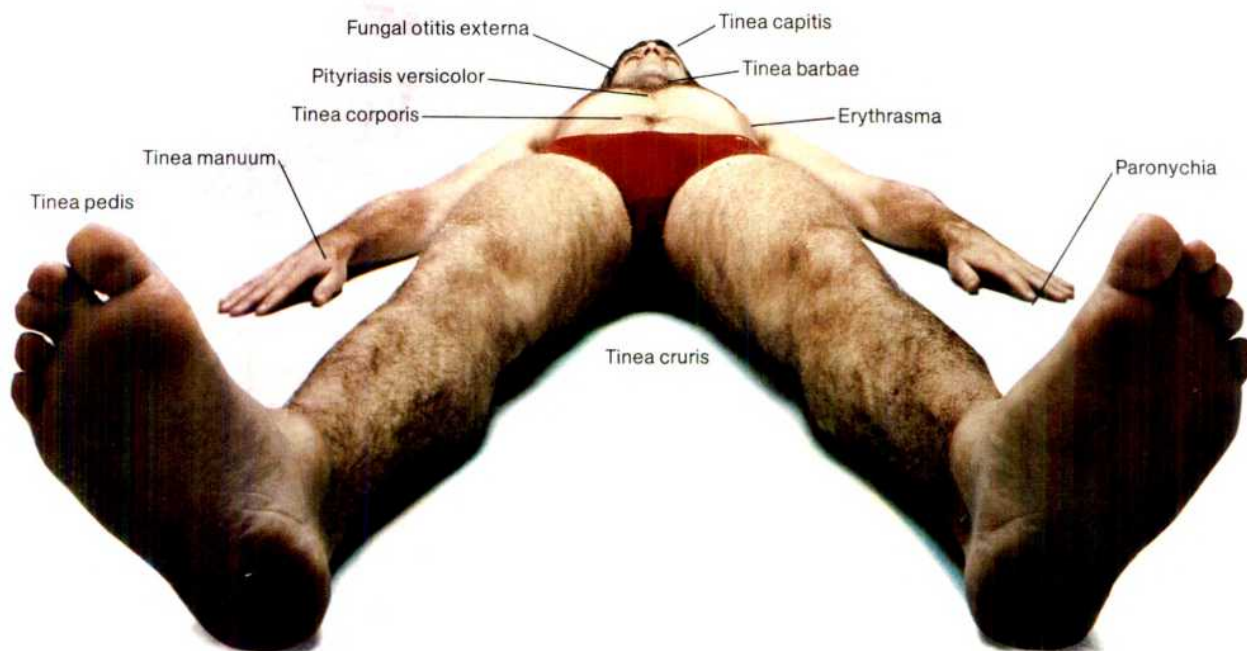
Glaxo

Leaders in topical steroid therapy

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Glaxo Laboratories Limited
Greenford, Middlesex UB6 0HE

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much more than a treatment for athlete's foot



Wherever fungal skin infection occurs – trunk, limbs, head, groin or other skin folds – and whatever the causative organism – candida, dermatophyte or other fungi – Canesten® is effective, combining the individual effectiveness of nystatin, tolnaftate and Whitfield's in a spectrum of

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effective in all fungal skin infections

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PUVA for Psoriasis

PUVA facilities for the treatment of psoriasis are now available to private patients who are referred by Consultant Dermatologists. The Waldmann PUVA 6000 unit, widely acknowledged as the best of its kind, is used. It allows wholebody treatment in the standing position.

A regime has been developed based on a London Teaching Hospital's experience and practice. The staff are professionally qualified and trained in the Photochemotherapy of psoriasis. Visits by Dermatologists are welcome by appointment.

An Honorary Medical Advisory Committee made up of four consultant dermatologists is supervising the professional standards and practice of the clinic. Dermatologists, who would like to know more about this facility, are asked in the first instance to contact:

The Physician-in-Charge,
Psoriasis UVA (Medical Referrals Only) Ltd.,
20 Harley Street, LONDON W.1.
Telephone 01-580 0875

Volume 1, Number 1, January 1979

Journal of Developmental Physiology

Edited by Colin T. Jones

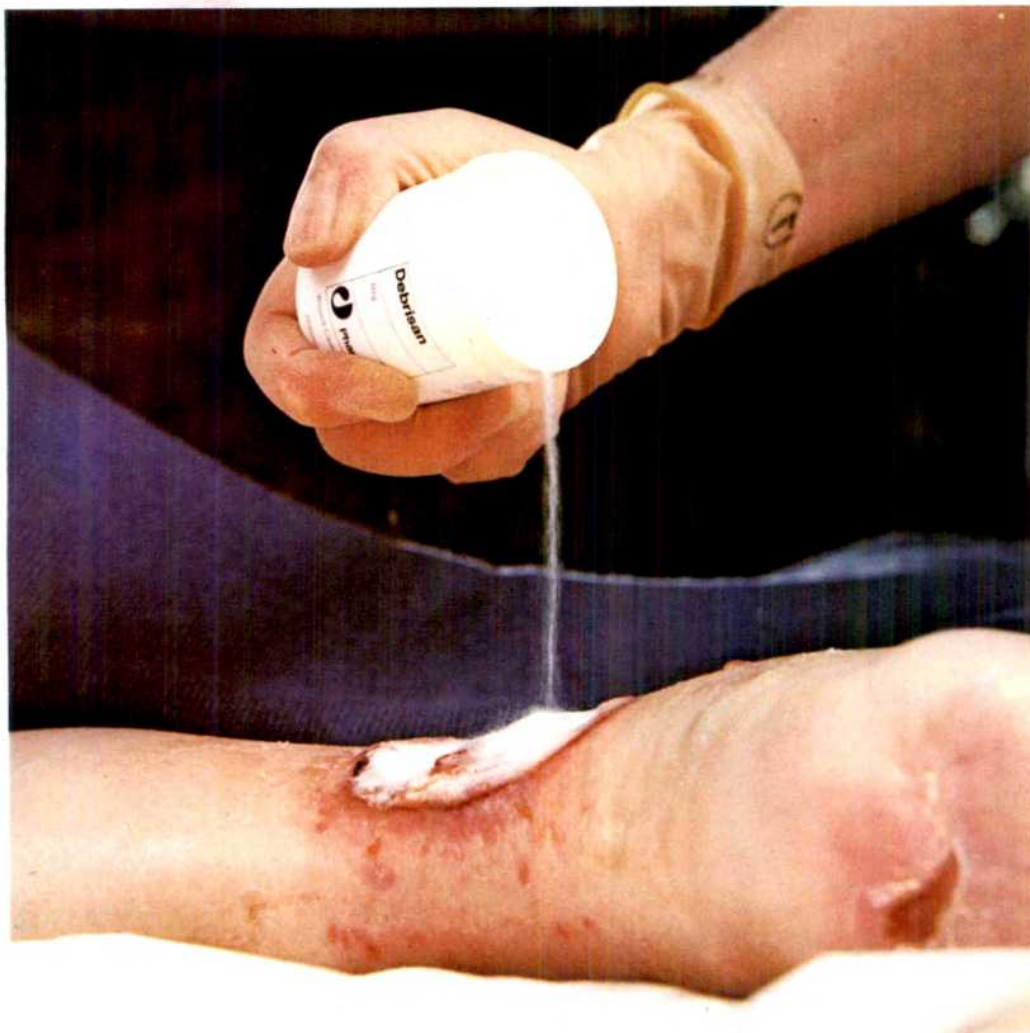
The Nuffield Institute for Medical Research, Headington, Oxford

The *Journal of Developmental Physiology* publishes papers describing the results of original research on any aspect (anatomical, biochemical, endocrine, pharmacological, physiological, etc) of the scientific study of the pregnancy, the foetus or the neonate of man or experimental animals. One of the major aims of the journal is to promote the communication of scientific information between individuals studying various aspects of development. Thus papers which clearly and succinctly report new observations and make their aims clear to scientists from a variety of disciplines are favoured. Papers will be considered on scientific merit alone and must report significant and carefully confirmed findings with full experimental details.

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Blackwell Scientific Publications Ltd
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Debrisan a major breakthrough in wound treatment



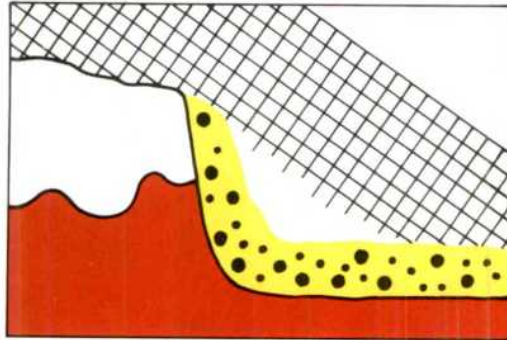
Full information available on request. Product Licence No. 0009/0021 Debrisan is a registered trade mark.
Debrisan is available as a castor containing 60g of sterile dextranomer.

Pharmacia (Great Britain) Ltd.,
Paramount House,
75 Uxbridge Road,
London W5 5SS.
Telephone: 01-579 0102/7



Conventional dressing

Passive * partial *
sometimes painful



Top and bottom left

A conventional dressing may bridge the wound and hold a layer of pus against the wound surface.

Red: Oedema

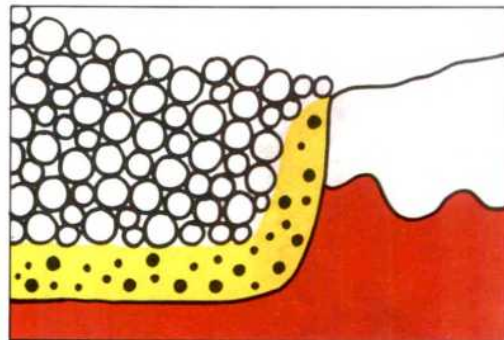
Yellow: Infected pus

Large black dots: Microorganisms and large molecules

Small black dots: Small molecules

Debrisan

active * continuous *
rapid relief of pain



Top right

Debrisan fills the wound achieving intimate contact with the wound surface.

Bottom right

Wound exudate is drawn into the Debrisan layer and away from the wound surface. Cleansing is continuous if Debrisan is replaced before saturation.

Debrisan consists of spherical beads of dextranomer 0.1-0.3mm in diameter which can suck up four times their own weight of moisture with considerable force. By continuous removal of bacteria and exudate by this means the wound is cleansed, often with the benefits of healing and decrease in malodour. Inflammation, oedema and crust formation are reduced. Grafts can often be made earlier through promotion of granulation tissue formation. Debrisan does not cause sensitisation and is thus of value in wounds such as leg ulcers where this is a major problem. Because of its mechanical mode of action Debrisan can be used to clear infected wounds prior to receipt of bacteriological reports. Early effective treatment with Debrisan can shorten the hospital stay to the benefit of all.

Indications

All kinds of secreting, suppurative wounds, such as post-operative wounds, leg ulcers, pressure sores, traumatic wounds, hand burns, suppurative ulcers, infected radio dermatitis, non-venereal penile ulcers.

Warning:

Debrisan spillage can render surfaces very slippery. Clear spillages promptly.

Caution:

In order to avoid cross-infection it is recommended that the contents of a castor be confined to the treatment of one patient only.

Debrisan-healing through cleansing



Eczema. Why Oilatum Emollient

After steroids have relieved the inflammation and pruritus, there is still an important stage of treatment — **healing the lesion**

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A study of lupus erythematosus with particular reference to generalized discoid lupus

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SUMMARY

The classification of lupus erythematosus (LE) is difficult because of variable and multisystem involvement, occurrence of transitional forms, and overlapping with connective tissue disorders. One of the earliest attempts at classification was made by O'Leary (1934), who classified LE clinically into four main types: (1) chronic discoid lupus erythematosus (DLE), or fixed type with the typical erythematous, scaly, well-demarcated eruption, usually showing follicular plugging and atrophy, confined to the head region; (2) generalized DLE, or chronic disseminated type differing from the localized discoid type in that the erythematous plaques are found not only on the head area but also below the neck region (O'Leary considered that constitutional systems were generally lacking in these two types); (3) subacute disseminated LE; and (4) acute disseminated LE. The last two categories reflect what we now call systemic lupus erythematosus (SLE) of varying severity.

Many authors have used O'Leary's clinical classification, but progress in laboratory medicine—in particular the discovery of the LE cell—has led to other schemes, the most widely accepted at present being that related to the American Rheumatism Association's (ARA) preliminary criteria for the diagnosis of SLE (Cohen *et al.*, 1971). These criteria have been criticized by many authorities because they do not include important data, such as those relative to the antinuclear antibody test, serum anti-native-DNA, serum complement levels, many neurological abnormalities, renal biopsy changes, and cutaneous immunofluorescent (IF) findings. However, Dubois (1974b) pointed out that all categorizations of LE are arbitrary, and he accepted the ARA criteria as a successful method for differentiating LE.

This report involves the clinical and laboratory findings in 123 patients with LE seen at the Mayo Clinic during the 3-year period 1971–1974 and an assessment of the validity and usefulness of O'Leary's classification.

MATERIALS AND METHOD

Detailed histories were obtained from 123 patients with LE. In addition, the following laboratory tests were performed: haemoglobin, leukocyte and differential counts, erythrocyte sedimentation

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rate (Westergren), LE clot test, antinuclear antibody test, serum creatinine, serum bilirubin, serum glutamic oxalacetic transaminase, lactic dehydrogenase, and urinalysis.

Serum levels of IgG, IgA, and IgM were measured in 80 patients, total serum complement in 95, serum C3 in 26, serum C4 in 31, antibodies to double-stranded DNA (anti-nDNA) in 69, and rheumatoid factor in 93. The Automated Reagin Test (ART) for syphilis was done in 76. Creatinine clearance and 24 h urine protein excretion were measured if proteinuria or an abnormal urinary sediment was found.

Punch biopsy specimens (4 mm) of lesional skin were taken from 98 patients after local anaesthesia with 1% lidocaine without epinephrine. The specimens were fixed in 10% formaldehyde and were stained with haematoxylin-eosin, resorcin-fuchsin-Giemsa, and Alcian blue-periodic acid-Schiff.

Punch biopsy specimens were also obtained for IF study from the lesional skin of 91 patients and from the uninvolved, sun-protected skin of 85 patients. All specimens were quick-frozen in liquid nitrogen, stored at -70°C , and processed within 1 week. Specimens were sectioned in a cryostat at -30°C and were used unfixed in a standard IF staining procedure (Beutner, Chorzelski & Jordon, 1970) with labelled antisera to IgG, IgA, IgM, C3, and fibrin. Fluorescein-to-protein ratios (F/P), units of antiserum, and use dilutions (UD) for these conjugated antisera have been reported previously (Beutner *et al.*, 1970). The presence of C1q, factor B, and properdin was determined by an indirect IF technique described by Provost & Tomasi (1973) and Jordon, Schroeter & Winkelmann (1975).

OUR SERIES

Initially, all cases were assessed for fulfilment of the ARA criteria of SLE, which correspond to O'Leary's types 3 and 4. The remaining cases were then placed in O'Leary's types 1 and 2, depending on extent of cutaneous involvement. Three major groups resulted: (1) DLE (23 cases), (2) generalized DLE (46 cases), and (3) SLE (54 cases). The last two groups were each analyzed both as total number of patients and as number of untreated patients, that is, patients who did not receive systemic therapy.

Patients with DLE. The 23 patients in this group had follow-up at the Mayo Clinic of from 0.2 to 24 years, with a median of 2.5 years. The median age at onset of DLE was 39 years, with a range from 7 to 65 years. Classical histological features of LE were present in lesional skin biopsy specimens taken on the initial visit. The LE clot test was repeatedly negative in all patients during the follow-up period, and none of the 23 patients had significant systemic symptoms. Three patients had been treated with antimalarial therapy, but all three were being treated with topical corticosteroid cream at the time of study and were advised to avoid sunlight. When examined, in none of the 23 patients had their disease progressed to generalized DLE or to SLE. Five of the patients (22%) had histories of drug allergy, and one had a cousin with SLE.

Patients with generalized DLE. These 46 patients had had DLE diagnosed on their first clinic examination and have subsequently been followed up from 0.16 to 42 years (median 3 years). Twenty-two patients (48%) had follow-up of 5 years or longer. At the time of review, in none of the patients with generalized DLE had the disease progressed to SLE. Classical histological features of LE were present at initial presentation in lesions of 36 of the 40 patients biopsied; two specimens showed poikiloderma and two showed perivascular dermal lymphocytic infiltrate. The ages of the patients at onset of eruption ranged from 6 to 67 years, with a median of 38.5 years. Examination revealed that the cutaneous involvement could be categorized by three general sites: (1) head, trunk, and limbs (15 patients), (2) head and upper trunk (19 patients), and (3) head and upper limbs (12 patients).

At the time of review, all 46 patients were being treated: 25 by local application of corticosteroid cream, 8 by antimalarial drugs, 9 by oral corticosteroid therapy (5-40 mg prednisone daily), and 4 by antimalarial drugs and oral corticosteroid therapy.

Six of the patients (13%) had histories of drug allergy. None had a positive family history of connective tissue disease.

Patients with SLE. Of the 54 patients with SLE, 11 had no cutaneous manifestations. Of the remaining 43 patients, 10 had generalized plaques, 9 had generalized erythema, 12 had urticaria, 3 had a generalized red papular eruption, 3 had DLE lesions on the face and arms, 2 had DLE lesions on the face, 1 had generalized livedo reticularis, 1 had generalized vasculitis, 1 had generalized purpura, and 1 had petechial lesions of the trunk. The median age at onset was 33 years (range 7-58 years), and the median duration of follow-up was 4.26 years, with a range of 0.25-24 years. Twenty patients were receiving immunosuppressive therapy when examined: 15 with oral prednisone, 2 with prednisone and azathioprine, 2 with melphalan, and 1 with azathioprine.

Six patients (11%) had histories of drug allergy. Three had positive family histories of connective tissue disease, two had sisters with SLE, and one had a sister with DLE.

RESULTS

There was a significantly higher percentage of females in the SLE group than in the other two groups, and 20% of the SLE group did not have any cutaneous eruption (Table 1). Systemic symptoms were absent in the DLE group and were uncommon in the generalized DLE group when comparison was made with the SLE group. Fever, pleurisy, splenomegaly, arthralgia, arthritis, and central-nervous-system abnormalities were much more common in the SLE group than in the other two groups. Interestingly, synovitis was noted in 41% of the SLE group, which contrasted with an incidence of 2% in the generalized DLE group. However, arthralgia alone was present in 33% of the SLE group

TABLE 1. Clinical features in 123 cases of lupus erythematosus

	Percent of cases				
	Generalized DLE			SLE	
	DLE (23 pts)	Total (46 pts)	Untreated (25 pts)	Total (54 pts)	Untreated (34 pts)
Female	61	57	48	91	82
Cutaneous lesions	100	100	100	80	79
Oral lesions	4	2	4	11	12
Scarring alopecia	4	4	8	13	14
Photosensitivity	74	87	60	73	70
Fever	0	4	4	41	32
Pleurisy	0	2	0	24	14
Splenomegaly	0	2	0	12	8
Arthralgia	0	15	8	33	32
Arthritis	0	2	0	41	44
Raynaud's phenomenon	0	11	0	18	21
Central-nervous-system abnormalities	0	0	0	19	14

TABLE 2. Laboratory data in 123 cases of lupus erythematosus

	Percent of cases				
	Generalized DLE			SLE	
	DLE (23 pts)	Total (46 pts)	Untreated (25 pts)	Total (54 pts)	Untreated (34 pts)
Anaemia	0	15	16	35	32
Leukopenia	13	14	8	30	29
ESR (mm in 1 h)					
> 20	35	60	52	89	88
> 50	4	14	16	65	62
> 75	0	7	8	41	41
Positive LE test	0	0	0	65	64
Positive ANA titre	29	49	40	79	79
$\geq$ 1:64	19	36	32	68	71
$\geq$ 1:128	0	13	12	45	38
$\geq$ 1:256	0	4	0	28	35
Positive anti-nDNA	0	0	0	64	66
Positive rheumatoid factor	0	6	6	6	3
False-positive ART test	5	4	7	17	15
Abnormal urine	0	0	0	52	72

and 15% of the generalized DLE group. Raynaud's phenomenon was not uncommon in SLE and was absent in DLE, but its occurrence in generalized DLE was confined to five patients who were receiving systemic treatment.

Anaemia was not encountered in DLE and was twice as common in SLE as in generalized DLE (Table 2). Leukopenia was a feature of all groups but was more common in the SLE group. The degree of elevation of the erythrocyte sedimentation rate and the magnitude of the antinuclear antibody titre were most striking in the SLE group, showing the lowest values in the DLE group and intermediate values in the generalized DLE group. Outstanding features were the absence of a positive LE test in both DLE groups and elevation of serum anti-nDNA binding in 22 of 35 patients with SLE but normality in 10 patients with DLE and in 22 patients with generalized DLE. The percentage of patients with positive rheumatoid factor did not differ strikingly among the groups, but patients with false-positive ART results were more frequent in the SLE group.

A finding of major significance was the absence of abnormal urine in the DLE groups; this contrasted with an incidence of 52% in the total group of patients with SLE and of 72% in the 34 patients with SLE who were not receiving systemic therapy. Of the 28 patients with SLE with abnormal urine, 24 were shown by creatinine clearance and 24-h urinary protein measurement to have significant renal dysfunction. This was confirmed by renal biopsy in 10 of the 24 patients. Creatinine clearance and 24-h urinary protein studies in 15 patients with generalized DLE did not show any renal disease.

Estimation of the three major immunoglobulin classes did not distinguish among the three groups, being elevated in many of the 80 patients tested (Table 3). However, serum IgM elevation was more common in SLE. Total serum complement level was depressed in half of the SLE group, in 14% of the generalized DLE group, and in none of the DLE group. Decreased levels of serum C₃ and C₄ were much more common in the SLE group than in the generalized DLE group.

TABLE 3. Immunological features in 80 cases of lupus erythematosus

Serum	DLE	Generalized DLE		SLE	
		Total	Untreated	Total	Untreated
Increased IgG	1/9 (11)*	3/30 (33)	1/14 (7)	13/41 (32)	13/30 (43)
Increased IgA	1/9 (11)	9/30 (30)	5/14 (20)	9/41 (22)	8/30 (27)
Increased IgM	3/9 (33)	10/30 (33)	3/14 (21)	20/41 (49)	20/30 (67)
Decreased CH ₅₀	0/12	5/35 (14)	3/18 (17)	26/48 (54)	16/20 (53)
Decreased C ₃	Not done	3/6 (50)	2/3 (67)	14/20 (70)	9/14 (64)
Decreased C ₄	Not done	1/6 (17)	0/2 (—)	13/31 (41)	6/16 (37)

* Percentages given within parentheses.

The IF findings at the dermal-epidermal junction and in the superficial and mid-dermal blood vessels of lesional skin are shown in Table 4. Nineteen (95%) of 20 patients with DLE showed positive IF staining at the dermal-epidermal junction, compared with 38 (93%) of 41 patients with generalized DLE and 29 (97%) of 30 patients with SLE. Positive staining for IgM, C₃, and C_{1q} was detected in a high percentage of all groups including untreated patients. Deposition of IgG and C₄ was more common in SLE. Surprisingly, fibrin deposition was more frequent in DLE and generalized DLE than in SLE. The deposition of factor B and properdin was noted in a small percentage of

TABLE 4. Direct IF study of involved skin in lupus erythematosus

Antisera	DLE	Generalized DLE		SLE	
		Total	Untreated	Total	Untreated
Dermal-epidermal junction					
IgG	2/10 (10)*	6/41 (15)	3/22 (14)	11/30 (37)	9/19 (47)
IgM	18/20 (90)	32/41 (78)	19/22 (86)	28/30 (93)	18/19 (95)
IgA	0/4	9/21 (43)	1/10 (10)	2/14 (14)	2/10 (20)
C3	14/20 (70)	25/40 (63)	15/21 (71)	24/29 (83)	15/19 (79)
C1q	4/5 (80)	7/9 (78)	5/5 (100)	16/16 (100)	10/10 (100)
C4	1/4 (25)	2/12 (17)	1/5 (20)	3/6 (50)	2/4 (50)
Fibrin	18/20 (90)	31/38 (82)	19/21 (90)	10/26 (38)	3/16 (19)
Factor B (C3PA)	0/3	2/11 (18)	2/6 (33)	4/17 (24)	2/9 (22)
Properdin	2/5 (40)	6/11 (54)	4/7 (57)	8/18 (44)	3/8 (38)
Blood vessel wall					
IgG	0/20	0/41	—	1/30 (3)	1/19 (5)
IgM	0/20	4/41 (10)	3/22 (14)	10/30 (33)	7/19 (37)
IgA	0/4	0/21	—	0/14	0/10
C3	0/20	0/40	—	6/29 (21)	6/19 (32)
C1q	0/5	0/9	—	2/16 (12)	1/10 (10)
C4	0/4	0/12	—	0/6	0/4
Fibrin	0/20	4/38 (11)	2/21 (10)	5/26 (19)	2/16 (12)
Factor B (C3PA)	0/3	0/11	—	0/17	0/9
Properdin	0/5	0/11	—	0/18	0/8

* Percentages given within parentheses.

TABLE 5. Direct IF study of uninvolved skin in lupus erythematosus

Antisera	DLE	Generalized DLE		SLE	
		Total	Untreated	Total	Untreated

Dermal-epidermal junction					
IgG	0/11	0/29	0/14	13/46 (28)	5/29 (17)
IgM	0/11	5/28 (18)*	4/13 (31)	42/46 (91)	28/29 (97)
IgA	0/2	0/13	0/8	2/20 (10)	1/11 (9)
C3	0/11	3/29 (10)	2/14 (14)	16/45 (36)	15/29 (52)
C1q	Not done	1/2 (50)	1/1 (100)	9/12 (75)	4/6 (66)
C4	Not done	0/8	0/3	1/8 (12)	1/4 (25)
Fibrin	0/10	3/27 (11)	1/13 (8)	17/40 (43)	3/29 (10)
Factor B (C3PA)	Not done	0/3	—	4/11 (36)	0/5
Properdin	Not done	0/3	—	4/11 (36)	0/4

Blood vessel wall					
IgG	0/11	0/29	0/14	0/46	0/29
IgM	0/11	4/28 (14)*	3/13 (23)	7/46 (15)	4/29 (14)
IgA	0/2	0/13	0/8	1/19 (5)	0/11
C3	0/11	2/29 (7)	1/14 (7)	4/45 (9)	2/29 (7)
C1q	Not done	0/2	0/1	3/12 (25)	1/6 (17)
C4	Not done	0/8	0/3	0/8	0/4
Fibrin	0/10	1/27 (4)	0/13	7/40 (18)	3/29 (10)
Factor B (C3PA)	Not done	0/3	—	0/11	0/5
Properdin	Not done	0/3	—	0/11	0/4

* Percentages given within parentheses.

patients. There were no significant differences in IF study results between treated patients and total patients in either of the patient groups.

Deposits were not found in blood vessels of involved skin in the DLE group. Of 41 patients with generalized DLE, 4 had positive IF of the upper and mid-dermal vessels with anti-IgM and 4 showed deposits of fibrin. There was IgM deposition in one-third of the SLE group, and a smaller percentage showed positive C3 and C1q staining.

The results of IF studies of uninvolved skin are summarized in Table 5. None of the patients with DLE who were tested had positive staining at the dermal-epidermal junction. Of 29 patients with generalized DLE, 5 (17%) showed positive staining at the dermal-epidermal junction. Of these five, all showed IgM deposition, three showed C3, three showed fibrin, and only one showed C1q. Staining at the dermal-epidermal junction was found in 44 of the 45 patients with SLE who were studied (98%). IgM and C1q were present in more than 90% of those tested. Factor B and properdin were found in trace amounts in about one-third of the patients studied. Positive dermal blood vessel fluorescence was not found in any of the 11 patients with DLE who were tested but was present in 4 (14%) of the patients with generalized DLE and in 14 (30%) of the 46 patients with SLE.

DISCUSSION

The relationship between DLE and SLE is controversial. Dubois (1974b) maintained that both are systemic disorders and form part of a disease spectrum. In his comprehensive textbook on LE, he

presented evidence supporting his contention and pointed out that both share many features, including the following: clinical and histological cutaneous changes; cutaneous blood vessel abnormalities in uninvolved sites; laboratory test abnormalities, including an elevated level of serum anti-nDNA antibody; evidence of glomerulonephritis on renal biopsy; immunoglobulin deposition at the dermal-epidermal junction of involved skin, as demonstrated by direct IF examination; an increased incidence of antibodies to viral and synthetic double-stranded RNA; cytoplasmic virus-like particles in involved skin. Dubois (1974a) estimated that approximately 10% of patients with DLE will eventually develop overt SLE, and similar values of 7.3% (Storck & Berzups, 1962) and 12% (Scott & Rees, 1959) have been reported in large series of DLE.

By contrast, others, such as Burch & Rowell (1970), on the basis of extensive analysis of age and sex distribution, contended that most patients with DLE, even those with haematological and serological abnormalities, will not have SLE and that they represent a distinct group with a different genetic background and a different natural history. Rowell (1971) believed that the risk of patients with DLE developing SLE is less than 5%, and in a 12-year follow-up of 120 patients with DLE, he found that SLE developed in only 2 (1.7%). SLE did not develop in any of the 364 patients with DLE whose records were reviewed by Lodin & Thyresson (1955), although 25% of them had had their disease for 5 years or longer. Similarly, Marten & Blackburn (1961) did not find evidence of SLE in any of their 51 patients with DLE at 5-year follow-up, and other reports of large series of patients with DLE have revealed a low incidence of such a progression—for example, 1.3% of 370 patients (Cannon & Curtis, 1958) and 0.76% of 585 patients (Herrmann, Koch & Höft, 1962).

Rothfield *et al.* (1963) discussed the problem of lack of control groups in most LE studies. They found that the only statistically significant differences between 65 patients with DLE and 65 normal controls were in loss of weight and in presence of leukopenia.

DLE. Our data regarding median age at onset, female preponderance, and incidence of photosensitivity agree with those of Dubois, but the lack of any systemic symptoms in our patients contrasts with the incidence of 62% in his 26 cases and of 45% among the 53 cases reported by Rothfield *et al.* (1963). Our data are more in agreement with Lodin & Thyresson (1955), who noted systemic symptoms in less than 2% of their 364 patients.

In contrast to what we observed, Dubois (1974a) found a higher incidence of the following: anaemia (12%), leukopenia (33%), elevated ESR (90%), positive LE test (8%), and abnormal urine (8%). The results of Rothfield *et al.* (1963) differed from ours mainly in the higher incidence of leukopenia (31%), the lower incidence of positive antinuclear antibody test (2%), and the positive LE clot test (2%). Lodin & Thyresson's (1955) 364 patients, in comparison with our series, had a higher incidence of anaemia (5.5%) and a lower incidence of leukopenia (2.2%). Only Dubois' patients had abnormal urine (8%).

The IF findings at the dermal-epidermal junction in our patients with DLE are similar to those noted in previous reports (Pohle & Tuffanelli, 1968; Baart de la Faille-Kuyper, 1969; Prystowsky, Herndon & Gilliam, 1976). We did not find any deposition of immunoglobulins in dermal blood vessels, in contrast to Baart de la Faille-Kuyper & Cormane (1968), who noted such deposits in 38% of involved skin and in 36% of uninvolved skin.

Generalized DLE. The marked variability of the results can be seen when our data are compared with those from previous reports of patients with generalized DLE. Montgomery & McCreight (1949) described a total of 103 patients seen at the Mayo Clinic: 80 patients were seen before 1938 and 23 were seen during the next 9 years. Data from the 80 patients resembled our data with regard to female preponderance, absence of central-nervous-system abnormalities, and incidences of fever,

anaemia, leukopenia, and false-positive serological test for syphilis. Photosensitivity was much less common, oral lesions were more common, and abnormal urine was noted in 3%. Data from the later series of 23 patients resembled those from our present series in that females predominated and central-nervous-system abnormalities were absent. However, photosensitivity was less common, and anaemia and leukopenia were twice as frequent. A major difference was the finding of abnormal urine in 39% in their series.

In Lodin & Thyresson's (1955) series of 94 patients, there was a female:male ratio of 2:1 and an age range at onset similar to that in our series. Fever was noted in 2% and arthralgia in 6%; and one patient had renal involvement. Anaemia was seen in 6.3% and leukopenia in 2%—a much lower incidence than that in our group. None of the 11 patients tested had a positive LE clot test, and 41% had an elevated erythrocyte sedimentation rate. No deaths occurred and no patient developed SLE—33% had had follow-up of 5 years or more—data that parallel the findings in this study.

The data from Marten & Blackburn's (1956) six cases resembled our findings and showed female preponderance, anaemia, and normal urine, but by contrast, the LE clot test was positive in two patients and fever and leukopenia were absent. The 15 patients in the series by Dubois & Martel (1956) differed significantly from ours because of higher incidence of the following factors: number of female patients (73%), mucous membrane involvement (13%), alopecia (33%), fever (27%), pleurisy (40%), Raynaud's phenomenon (20%), anaemia (60%), leukopenia (47%), positive LE clot test (7%), positive serological test for syphilis (7%), and abnormal urine (7%). Of Kushniruk's (1957) 5 patients (3 females), all were photosensitive and had Raynaud's phenomenon, 3 were anaemic, 4 had leukopenia, and 2 had abnormal urine. None had a positive LE clot reaction. Of 12 patients reported by Rothfield *et al.* (1963), 2 had pleurisy, 1 had leukopenia, 2 had a positive LE clot reaction, 2 had a positive serological test for syphilis, and 1 had abnormal urine.

Most series did not distinguish between arthritis and arthralgia, and the incidence of these combined findings ranged from 20 to 67%. Of Kushniruk's (1957) 5 patients, 2 had arthritis and 2 had arthralgia. In all series except one (Lodin & Thyresson, 1955), more than half of the patients had an elevated erythrocyte sedimentation rate.

The ANA test was performed in Rothfield's 12 cases and was positive in 1 case. Kalsbeek & Cormane (1967) reported that the LE clot test was negative in their 12 patients, whereas the ANA test was positive in 3. In the 12 patients, direct IF procedure showed immunoglobulin deposition at the dermal-epidermal junction in lesional skin, whereas only 3 patients had a similar finding in uninvolved skin.

In LE, there is controversy regarding the ideal site for taking biopsy specimens of uninvolved skin for direct IF (Monroe, 1977). When cutaneous lesions are absent, most authors recommend sun-exposed skin, whereas light-protected sites are recommended when differentiating SLE from DLE. Several studies have clearly shown that direct IF from uninvolved light-protected skin correlates with laboratory measurements that have been associated with disease activity and poorer prognosis (Monroe, 1977).

Five patients (3 females and 2 males) had positive IF staining at the dermal-epidermal junction of uninvolved light-protected skin. Of these, one—a 16-year-old patient—was followed up for 1 year and the other 4—with ages ranging from 43 to 49 years—were followed up for more than 10 years. Of the 5, 3 had generalized lesions and 2 had involvement of head and arms. Only one was symptomatic (Raynaud's phenomenon); 3 had positive ANA (titre equal to or less than 1:64), and all had negative anti-nDNA.

Two patients had low total serum complement levels with normal serum C4. Despite clinical improvement with antimalarial therapy, these 2 patients had no change in laboratory data after 1 year.

The lesions of these 5 patients probably represent a transition stage to SLE. In a separate study

of 17 patients with generalized DLE, Tappeiner, O'Loughlin & Jordon (in press) found elevated circulating immune complexes in one patient—the only patient to have positive IF staining at the dermal-epidermal junction of uninvolved skin. None of 5 patients with DLE had elevated levels, in contrast to 13 of 16 patients with SLE.

In a detailed clinical and laboratory study of 80 selected asymptomatic and untreated patients with chronic cutaneous LE, Prystowsky, Herndon & Gilliam (1976) found that 34 (42%) had lesions both above and below the neck. Only a small percentage had any laboratory abnormalities, and none of the 31 patients tested had IF staining of normal skin at the dermal-epidermal junction. Comparison with our patients is difficult because the patients with generalized disease were not discussed separately, and all were asymptomatic. The LE test and anti-nDNA tests were negative, and none of the 61 patients followed up for longer than 3 years developed SLE.

SLE. The clinical findings in SLE in our series generally were similar to those reported by Dubois (1974a), except for a higher incidence of photosensitivity in our patients (73% vs 33%) and a lower incidence of fever (41% vs 84%) and pleurisy (24% vs 45%). We found objective clinical evidence of arthritis in 41% of the patients with SLE, which contrasts with the 76% reported in a review of 25 patients (Labowitz & Schumacher, 1971). Our laboratory data also were similar to Dubois' results except for a lower incidence of anaemia in our group (35% vs 56%), and to the data from Rowell's (1971), 40 patients except for a lower incidence of positive rheumatoid factor in our series (6% vs 37%).

In our 41 patients in whom serum immunoglobulin studies were performed, there were elevated IgM levels in one-half and elevated IgG levels in one-third. In a study of 26 patients with SLE, Cass *et al.* (1968) found that only serum IgG was significantly elevated, whereas in a study of 106 patients, Alarcón-Segovia & Fishbein (1972) found significant elevations of serum IgG in 49% and significant elevations of IgM in 71%. The finding of elevated serum IgM levels in two-thirds of our 30 patients with SLE who were untreated is interesting in that IgM was the major immunoglobulin found at the dermal-epidermal junction or the dermal blood vessels.

The IF finding at the dermal-epidermal junction resembles previous data reported from our clinic (Jordon, Schroeter & Winkelmann, 1975) in that IgM and C1q and C3 were the most common deposits in involved skin and IgM and C1q in uninvolved skin; but C3 deposition in uninvolved skin was less common (36% vs 83%). Factor B (C3PA) and properdin were found in a smaller percentage of cases in the present series. IgM has been the predominant immunoglobulin deposited at the dermal-epidermal junction of involved and uninvolved skin in previous studies (Pohle & Tuffanelli, 1968; Ullman, Halberg & Wolf-Jürgensen, 1975). In our series, the frequency of properdin deposition in lesional skin was similar to that noted by Schrager & Rothfield (1976), but such deposition was less frequently found in uninvolved skin.

The finding of immunoglobulins, predominantly IgM, and complement components on IF examination of dermal blood vessels in 33% of the involved and in 30% of the uninvolved skin contrasts with the results of other investigators, who found that 19 of 20 patients with SLE (95%) had such deposition in blood vessels of uninvolved skin (Baart de la Faille-Kuyper & Cormane, 1968).

CONCLUSIONS

Analysis of the clinical and laboratory data on 123 patients demonstrated the usefulness of O'Leary's 1934 clinical classification of lupus erythematosus (LE) syndromes into localized discoid LE, generalized discoid LE, and systemic LE. Although LE is a spectrum with overlap and transition occurring among the subgroups, our data suggest that the clinical and laboratory findings can distinguish the groups and that the transition is uncommon. Systemic complaints were absent in patients with

discoid LE and were unusual in patients with generalized discoid LE. In generalized LE, arthritis was very rarely seen, and in both forms of discoid LE, abnormalities of the central nervous system were never seen. The laboratory tests that were most helpful in distinguishing the subgroups were the LE test, anti-native-DNA, serum complement, and urinalysis. Positive direct immunofluorescence of uninvolved unexposed skin was uncommon in generalized discoid LE, and these cases may represent transition to systemic LE.

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REFERENCES

- ALARCÓN-SEGOVIA, D. & FISHBEIN, E. (1972) Serum immunoglobulins in systemic lupus erythematosus. *Clinical Science*, **43**, 121.
- BAART DE LA FAILLE-KUYPER, E.H. (1969) Lupus erythematosus: an immunohistochemical and clinical study of 485 patients. Thesis, Grafisch Bedrijf Schotamus and Jens, Utrecht, N.V.
- BAART DE LA FAILLE-KUYPER, E.H. & CORMANE, R.H. (1968) The occurrence of certain serum factors in the dermal-epidermal junction and vessel walls of the skin in lupus erythematosus and other (skin) diseases. *Acta dermato-venereologica*, **48**, 578.
- BEUTNER, E.H., CHORZELSKI, T.P. & JORDON, R.E. (1970) *Autosensitization in Pemphigus and Bullous Pemphigoid*. Charles C. Thomas, Springfield, Illinois.
- BURCH, P.R.J. & ROWELL, N.R. (1970) Lupus erythematosus: analysis of the sex- and age-distribution of the discoid and systemic forms of the disease in different countries. *Acta dermato-venereologica*, **50**, 293.
- CANNON, E.F. & CURTIS, A.C. (1958) A survey of lupus erythematosus in the University of Michigan Hospital since 1948. *Archives of Dermatology*, **78**, 196.
- CASS, R.M., MONGAN, E.S., JACOX, R.F. & VAUGHAN, J.H. (1968) Immunoglobulins G, A, and M in systemic lupus erythematosus: relationship to serum complement titer, latex titer, antinuclear antibody, and manifestations of clinical disease. *Annals of Internal Medicine*, **69**, 749.
- COHEN, A.S., REYNOLDS, W.E., FRANKLIN, E.C., KULKA, J.P., ROPES, M.W., SHULMAN, L.E. & WALLACE, S.L. (1971) Preliminary criteria for the classification of systemic lupus erythematosus. *Bulletin on the Rheumatic Diseases*, **21**, 643.
- DUBOIS, E.L. (1974a) The relationship between discoid and systemic lupus erythematosus. In: *Lupus Erythematosus: A Review of the Current Status of Discoid and Systemic Lupus Erythematosus and Their Variants* (Ed. by E.L. Dubois), 2nd edn, p. 459. University of Southern California Press, Los Angeles.
- DUBOIS, E.L. (1974b) Differential diagnosis, criteria for diagnosis, and classification of systemic lupus erythematosus (supplement). In: *Lupus Erythematosus: A Review of the Current Status of Discoid and Systemic Lupus Erythematosus and Their Variants* (Ed. by E.L. Dubois), 2nd edn, p. 534. University of Southern California Press, Los Angeles.
- DUBOIS, E.L. & MARTEL, S. (1956) Discoid lupus erythematosus: an analysis of its systemic manifestations. *Annals of Internal Medicine*, **44**, 482.
- HERRMANN, W.P., KOCH, H. & HÖFT, G. (1962) Katamnestiche Untersuchungen über den Verlauf des chronischen Erythematoses. *Hautarzt*, **13**, 309.
- JORDON, R.E., SCHROETER, A.L. & WINKELMANN, R.K. (1975) Dermal-epidermal deposition of complement components and properdin in systemic lupus erythematosus. *British Journal of Dermatology*, **92**, 263.
- KALSBECK, G.L. & CORMANE, R.H. (1967) The occurrence of immunoglobulins in the dermo-epidermal junction of the skin in lupus erythematosus and related syndromes. *Dermatologica*, **135**, 205.
- KUSHNIRUK, W. (1957) Systemic involvement in chronic lupus erythematosus. *Canadian Medical Association Journal*, **76**, 184.
- LABOWITZ, R. & SCHUMACHER, H.R., JR. (1971) Articular manifestations of systemic lupus erythematosus. *Annals of Internal Medicine*, **74**, 911.
- LODIN, A. & THYRESSON, N. (1955) Discoid and disseminated lupus erythematosus: cases observed at the Dermatological Clinic of Karolinska Sjukhuset between 1948 and 1954. *Acta dermato-venereologica*, **35**, 429.

- MARTEN, R.H. & BLACKBURN, E.K. (1956) Lupus erythematosus: clinical and hematological studies in seventy-seven cases. *Archives of Dermatology*, **73**, 1.
- MARTEN, R.H. & BLACKBURN, E.K. (1961) Lupus erythematosus: a five-year follow-up of 77 cases. *Archives of Dermatology*, **83**, 430.
- MONROE, E.W. (1977) Lupus band test. *Archives of Dermatology*, **113**, 830.
- MONTGOMERY, H. & MCCREIGHT, W.G. (1949) Disseminate lupus erythematosus. *Archives of Dermatology*, **60**, 356.
- O'LEARY, P.A. (1934) Disseminated lupus erythematosus. *Minnesota Medicine*, **17**, 637.
- POHLE, E.L. & TUFFANELLI, D.L. (1968) Study of cutaneous lupus erythematosus: by immunohistochemical methods. *Archives of Dermatology*, **97**, 520.
- PROVOST, T.T. & TOMASI, T.B., JR (1973) Evidence for complement activation via the alternate pathway in skin diseases. I. Herpes gestationis, systemic lupus erythematosus, and bullous pemphigoid. *Journal of Clinical Investigation*, **52**, 1779.
- PRYSTOWSKY, S.D., HERNDON, J.H., JR & GILLIAM, J.N. (1976) Chronic cutaneous lupus erythematosus (DLE)—a clinical and laboratory investigation of 80 patients. *Medicine (Baltimore)*, **55**, 183.
- ROTHFIELD, N., MARCH, C.H., MIESCHER, P. & McEWEN, C. (1963) Chronic discoid lupus erythematosus: a study of 65 patients and 65 controls. *New England Journal of Medicine*, **269**, 1155.
- ROWELL, N.R. (1971) Laboratory abnormalities in the diagnosis and management of lupus erythematosus. *British Journal of Dermatology*, **84**, 210.
- SCHRAGER, M.A. & ROTHFIELD, N.F. (1976) Clinical significance of serum properdin levels and properdin deposition in the dermal-epidermal junction in systemic lupus erythematosus. *Journal of Clinical Investigation*, **57**, 212.
- SCOTT, A. & REES, E.G. (1959) The relationship of systemic lupus erythematosus and discoid lupus erythematosus: a clinical and hematological study. *Archives of Dermatology*, **79**, 422.
- STORCK, H. & BERZUPS, S. (1962) Über Lupus erythematosus unter besonderer Berücksichtigung des Überganges von lokalisierten in generalisierte Formen. *Dermatologica (Basel)*, **124**, 142.
- TAPPEINER, G., O'LOUGHLIN, S. & JORDON, R.E. (1978) C1q binding activity in lupus erythematosus: correlation with the lupus band test. *Journal of Investigative Dermatology*, in press.
- ULLMAN, S., HALBERG, P. & WOLF-JÜRGENSEN, P. (1975) Deposits of immunoglobulins and complement C3 in clinically normal skin of patients with lupus erythematosus. *Acta dermato-venereologica*, **55**, 109.

Immunoelectronmicroscopic examination of early lesions in histamine induced immune complex vasculitis in man

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SUMMARY

In 4 cases of allergic vasculitis circulating immune complexes (IC) were demonstrated. Spontaneous and histamine induced vascular changes were studied by immunofluorescence microscopy. The early events in IC vasculitis were investigated at the ultrastructural level by immunoelectronmicroscopy using the peroxidase-antiperoxidase multistep technique.

Our findings support the concept that human IC vasculitis is triggered by the deposition of circulating IC in the walls of postcapillary venules between endothelial cells, pericytes and the layers of the basal lamina. Tissue destruction is only secondary due to local complement activation and the release of lysosomal enzymes from chemotactically attracted leukocytes.

According to the current concept, which is mostly supported by immunofluorescent findings (Parish & Rhodes, 1967; Parish, 1973; Schroeter *et al.*, 1971; Sams *et al.*, 1975; Gianetti, Serri & Bernasconi, 1976), immune complex vasculitis is triggered by the deposition of circulating immune complexes in vessel walls. The initial step of amplification is activation of the complement cascade with the release of anaphylatoxins C3a and C5a chemotactic for polymorphonuclear leukocytes. Inflammatory vascular damage and secondary tissue destruction are brought about by the disintegration of the accumulated neutrophils and the local release of hydrolytic lysosomal enzymes—a process which is well known from classical histopathology as leukocytoclasia.

The *late events* of leukocytoclastic vasculitis have already been studied in detail at the ultrastructural level in patients (Braverman & Yen, 1975; Perrot *et al.*, 1971; Ruiter & Molenaar, 1970) and in experimental model systems (Cochrane & Janoff, 1974). However, *initial events* such as the mechanism of immune complex deposition and the ultrastructural localization of immune complexes in the

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vessel walls have not yet been investigated by means of immunoelectronmicroscopy. Biopsies taken from macroscopically visible skin lesions in patients with allergic vasculitis usually show only the later stages of vascular disorder. At this time the noxious immune complexes have already been removed by chemotactically attracted polymorphonuclear leukocytes (Cream, Bryceson & Ryder, 1971). Therefore, vascular deposition of circulating immune complexes in clinically uninvolved skin of patients with allergic vasculitis was induced by intradermal injection of histamine according to Braverman & Yen (1975), Gower *et al.* (1976) and Sams *et al.* (1976). To our knowledge, this is the first immunoelectronmicroscopic report on *early events* in histamine induced human immune complex vasculitis.

MATERIAL AND METHODS

Case reports

All 4 patients had clinically typical allergic vasculitis confirmed by histopathology.

Case 1. E.P., 42-year-old man. Within 2 weeks, macules and slightly palpable purpuric papules developed, beginning at the ankles and lower legs, spreading to the lower parts of the trunk. A few days earlier, the patient had had a cold and had taken different analgesic drugs.

Case 2. A.G., 70-year-old man. For 4 months, irregular, partly gyrate, infiltrated purpuric erythematous lesions developed on the legs and the lower parts of the trunk.

Case 3. G.S., 46-year-old man. A tonsillitis was treated with penicillin and a combination of analgesic drugs. Three days later, an exanthem developed covering almost the entire body predominantly on the back and the lower parts of the extremities. The lesions were purpuric, flat macules or slightly palpable small nodules.

Case 4. E.K., 71-year-old man. The skin disease developed over a period of 3–4 months beginning on the back. The lower parts of the trunk and of the legs showed clinically the Schönlein–Henoch type of purpura, other areas resembled the Schamberg type.

Detection of circulating immune complexes

In all cases, circulating immune complexes were demonstrated by means of the ^{125}I -C1q deviation test according to the technique of Sobel, Bokisch & Müller-Eberhard (1975).

Experimental induction of vasculitis

In all cases, 0.1 ml of histamine (1:10,000) was injected intradermally into clinically uninvolved skin. The biopsy was taken 1 h (Cases 1, 3, 4) or 3 h (Case 2) after the injection and processed for immunofluorescence and immunoelectronmicroscopy.

Immunofluorescence

In all cases, biopsies from clinical lesions as well as from histamine-induced vasculitis were examined by direct immunofluorescence using FITC labelled antisera (see Table 1).

Immunoelectronmicroscopy

The method followed was approximately that of Holubar *et al.* (1975). The biopsy material was cut into small pieces of about 1 mm² and put in PBS with 6% glucose (pH 7.2). In Case 1 only, the tissue was fixed in 1% glutaraldehyde in PBS (pH 7.2) containing 0.1 M sucrose at 4°C, rinsed in

TABLE 1. Summary of serological, immunofluorescent and immunoelectronmicroscopic findings in 4 patients with allergic vasculitis

Test ¹²⁵ I-C1q-deviation test*	Case 1 (P.E.) 17.6%		Case 2 (G.A.) 44.0%		Case 3 (S.G.) 38.2%		Case 4 (K.E.) 32.5%	
	Before histamine	After injection	Before histamine	After injection	Before histamine	After injection	Before histamine	After injection
Immunofluorescence microscopy:								
FITC-Anti-IgG	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
FITC-Anti-IgM	Ø	Ø	Ø	+	Ø	+	Ø	+
FITC-Anti-IgA	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
FITC-Anti-C3	+	+	+	+	+	+	+	+
FITC-Anti-C4	Ø	Ø	+	+	Ø	+	Ø	+
FITC-Anti-C1q	Ø	Ø	+	+	Ø	+	Ø	+
FITC-Anti-C3 Activator	Ø	Ø	Ø	Ø	Ø	+	Ø	+
FITC-Anti-Fibrinogen	n.d.	n.d.	+	Ø	+	Ø	+	Ø
Immunoelectronmicroscopy:								
Anti-IgG		n.d.		+		+		+
Anti-IgM		n.d.		n.d.		+		+
Anti-C3		+		+		+		+

* Normal range of ¹²⁵I-C1q-deviation: 0-5%.
n.d. not done.

PBS-sucrose and then incubated for 1 h each step. In Cases 2, 3 and 4 fixation of any kind was omitted at this stage, and the incubation was 40 min each step. The following incubation sequence was performed at room temperature:

- (1) Forty min in rabbit antihuman C3 antibodies (dilution 1:10 or 1:20), Behringwerke Marburg/Lahn, Germany. Material was also incubated for 40 min in rabbit antihuman IgG (diluted 1:10 or 1:20) in Cases 2-4 and in rabbit antihuman IgM (diluted 1:10) in Cases 3 and 4.
- (2) Forty min in goat antirabbit IgG antibodies (dilution 1:2), Hyland, U.S.A.
- (3) Forty min in rabbit antihorseradish (HRP) antibodies (dilution 1:10), Dakopatts, Denmark.
- (4) Forty min in HRP (60 µg/ml), Miles, England.

The specimens were rinsed for 2 × 15 min in PBS after each incubation step. The sera were diluted with PBS containing 4% bovine albumin. After completion of these steps, the specimens were fixed for 1 h in 4% glutaraldehyde in PBS pH 7.2 at 4°C (Cases 1 and 2) or in Karnovsky's fixative at room temperature (Cases 3 and 4). The tissue was then rinsed overnight in PBS at 4°C (Cases 1 and 2), or after a short rinse (Cases 3 and 4), incubated in the Graham-Karnovsky medium for demonstration of HRP. The incubation time was between 30 and 45 min. After an additional short rinse in Tris-HCl buffer at pH 7.6 (Cases 1 and 2) or PBS (Cases 3 and 4) the material was postfixed in OsO₄ at 4°C. Dehydration and embedding in epon followed routine methods. Semi-thin sections were stained with methylene blue-azure II, and selected areas were cut with diamond knives on the Reichert OmU2 or OmU3 ultramicrotome. The sections were poststained with uranyl acetate and lead citrate and studied with a Philips EM 300.

In control preparations, the second incubation step was omitted.

RESULTS

Circulating immune complexes

In all 4 cases, a rather high titre of circulating immune complexes was demonstrated (see Table 1).

Immunofluorescence

In lesional skin the presence of C3 and fibrin was revealed in the walls and around affected vessels. In no instance were immunoglobulins observed. In histamine-induced lesions, C3 was positive in all 4 cases and IgM in 3 of the 4 cases. IgG was negative in all cases (for details, see Table 1).

Immunoelectronmicroscopy

Only histamine-induced skin reactions were examined. The preservation of the tissue was good. There were only slight signs of endothelial swelling which may in part be due to the process of vasculitis itself. The peroxidase-positive granules which marked the ultrastructural location of immunoglobulin deposition were easily identified on the basis of their density. No such granules were observed in the control sections. In all 4 patients, the ultrastructural findings were almost identical. Prefixation as done in Case 1 did not result in better tissue preservation and was therefore omitted from the following cases. The precipitates were detected within the walls of postcapillary venules. The examination of complement C3 gave the most characteristic pictures (Figs 1-3). Large amounts of small peroxidase positive granules were visible between individual endothelial cells, on

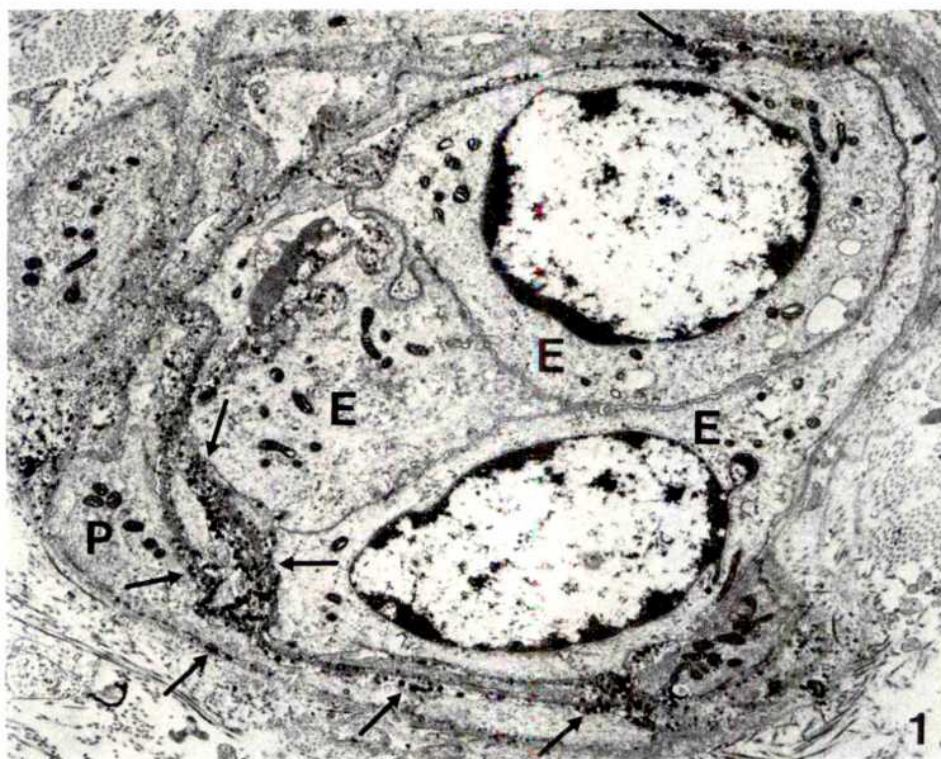


FIGURE 1. Postcapillary venule 3 h after histamine injection in normal skin of a patient with allergic vasculitis (Case 2). Immunoelectronmicroscopic demonstration of complement C3 deposits (arrows). E, endothelial cells; P, pericyte ($\times 9600$).

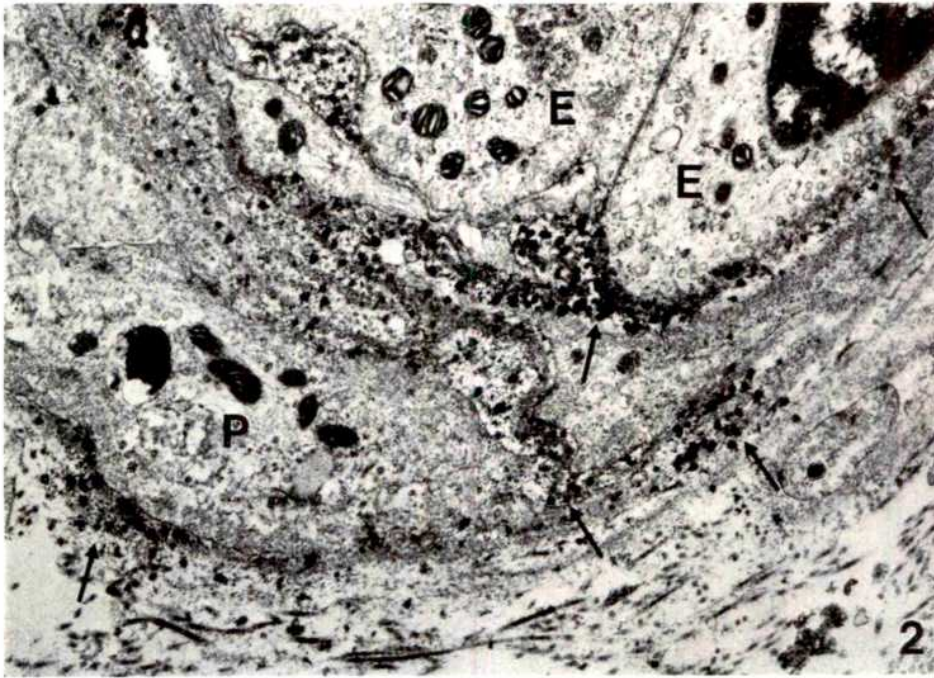


FIGURE 2. Immunoelectronmicroscopic demonstration of C3 (arrows) between endothelial cells (E) and pericytes (P) of a venule ($\times 18,400$).

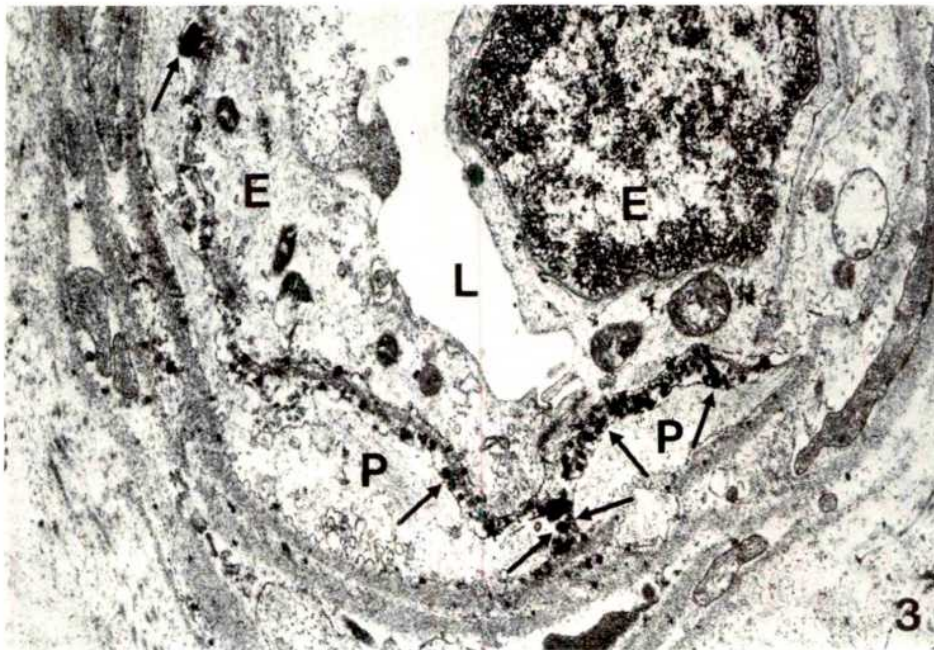


FIGURE 3. Immunoelectronmicroscopic demonstration of C3 1 h after histamine injection (Case 4). L, lumen; E, endothelial cells; P, pericytes ($\times 18,400$).

their outer surface below the basal lamina and between the basal lamina and the pericytes (Fig. 2). Granules were also found between the pericytes and the layers of the multiplied basal lamina surrounding the vessels (Fig. 3). Only small amounts of peroxidase positive material were present in the vicinity of the vessels.

The localization of IgM was very similar. However, fewer precipitates were seen, and the deposits sometimes formed large aggregates (Fig. 4). Though immunofluorescence was negative for IgG we were able to demonstrate small amounts of this immunoglobulin by immunoelectronmicroscopy in all 3 cases examined (Fig. 5).

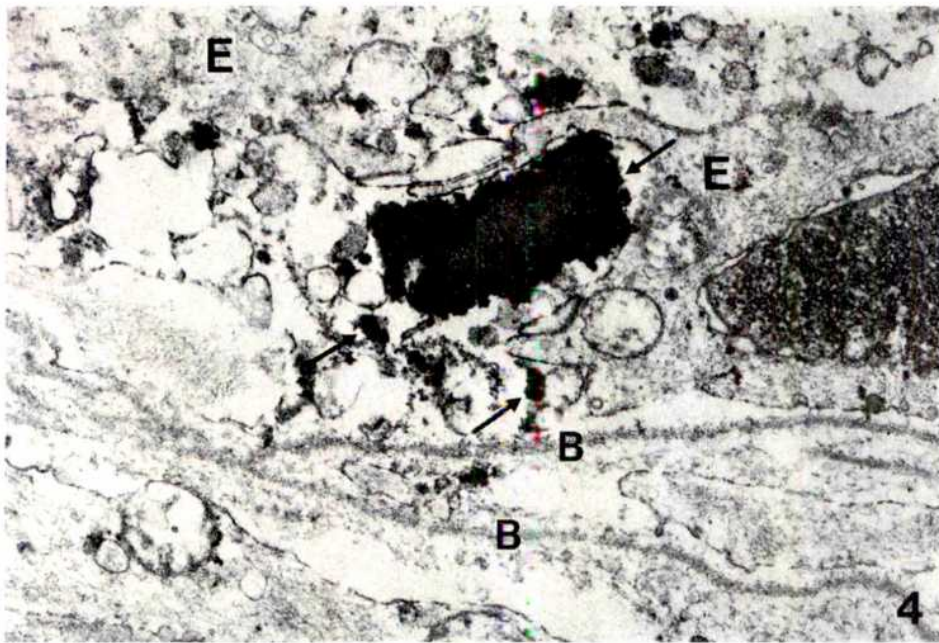


FIGURE 4. Immunoelectronmicroscopic demonstration of large aggregates of IgM (arrows) between endothelial cells (E) and the layers of the basal lamina (B) of a venule 1 h after histamine injection (Case 3) ($\times 14,500$).

These immunoprecipitates were found predominantly in areas without any accumulations of inflammatory cells, fibrin, or distinct signs of tissue damage. In some places, however, a cellular infiltrate was observed consisting of intact or disintegrating neutrophils, of macrophages and lymphocytes. Occasionally, B lymphocytes were recognizable by the presence of immunoglobulins on their surface (Fig. 6). Also, macrophages with C3 reactive material on their surface were detected. The macrophages seemed to ingest this material. In rare instances, macrophages and lymphocytes (probably T) were seen in close contact (Fig. 7).

DISCUSSION

The purpose of our study was to examine the following problems:

- (i) Localization of immunoglobulins and complement in an early stage of human vasculitis at the ultrastructural level.

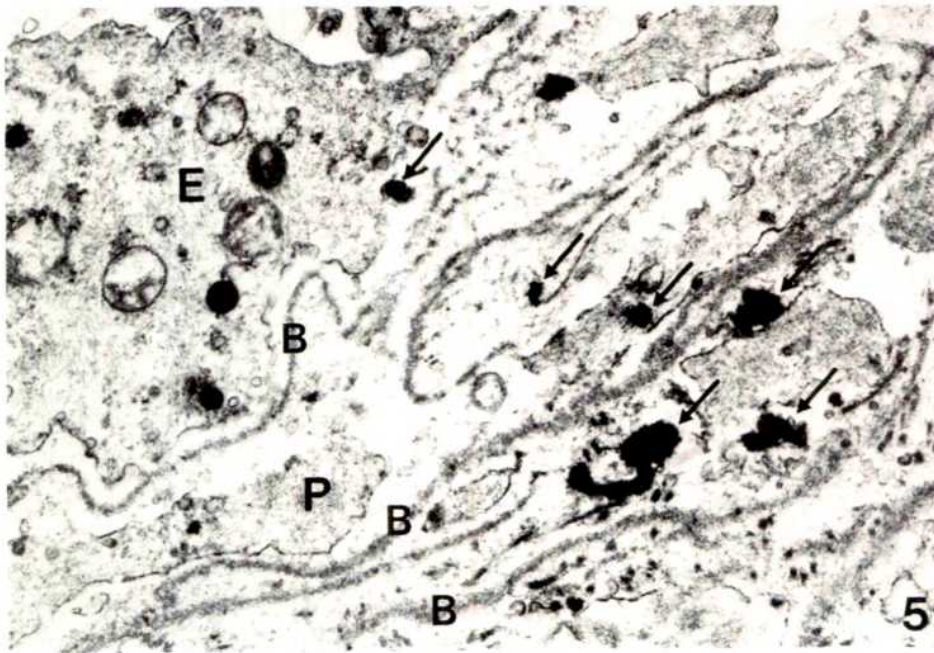


FIGURE 5. Demonstration of IgG (arrows) in the wall of a venule 1 h after histamine injection. Immunoelectronmicroscopy (Case 4). E, endothelial cell; P, pericyte; B, basal lamina ($\times 22,000$).

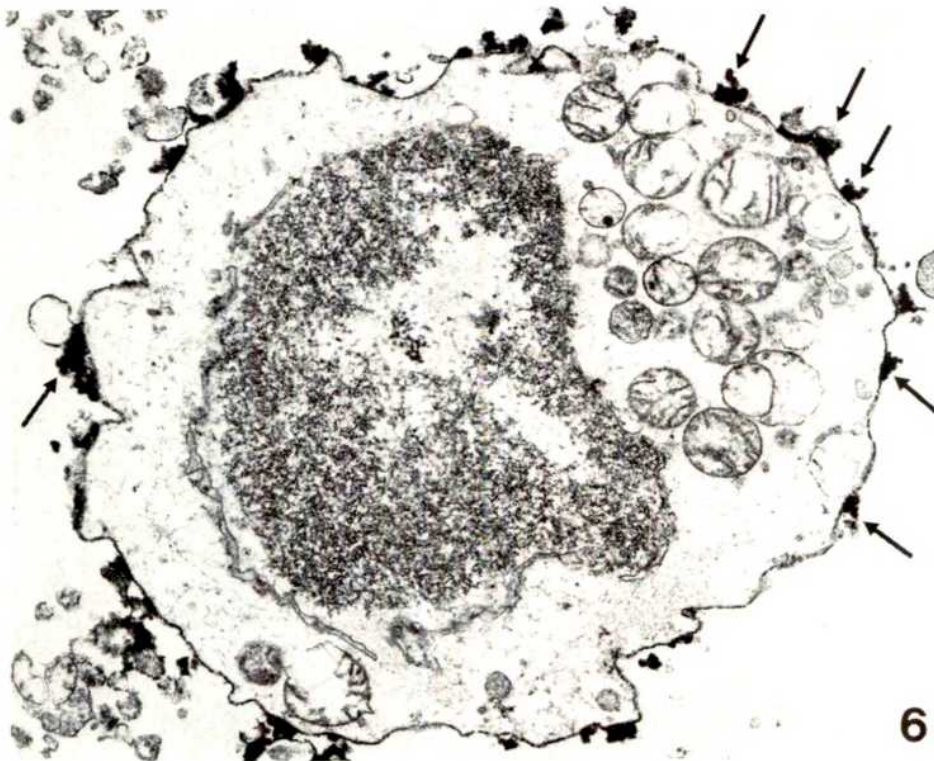


FIGURE 6. Tissue lymphocyte showing the presence of IgM on the surface (arrows). Immunoelectronmicroscopy (Case 3) ($\times 18,400$).

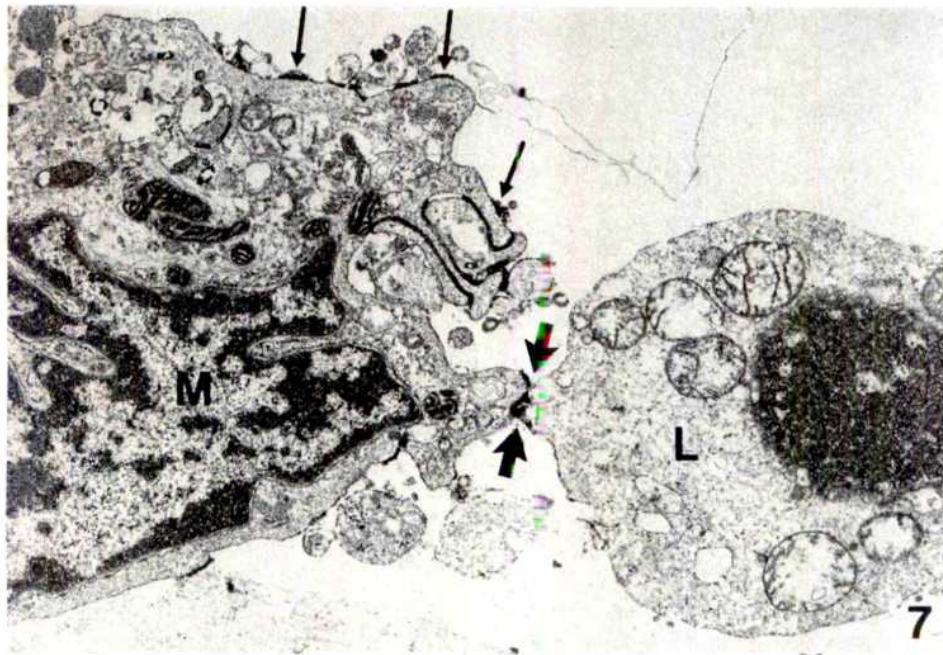


FIGURE 7. Macrophage (M) with the adherence of C3 reactive material on the surface (arrows) in close contact with a lymphocyte (L). Immunoelectronmicroscopy (Case 3) ($\times 14,500$).

(ii) Confirmation of the primary role of immune complex deposition in the sequence of events resulting in leukocytoclastic vasculitis.

In 4 patients with clinically and histologically typical allergic vasculitis, circulating soluble immune complexes (IC) have been demonstrated in the serum.

Circulating IC have been detected in a great number of different dermatological disorders including such unrelated diseases as bullous pemphigoid and psoriasis (Braun-Falco, Männel & Scherer, 1977). However, only in rare instances, such as allergic vasculitis, could the pathogenic significance of these circulating antigen-antibody complexes be established (Jones *et al.*, 1976). The ability to induce vascular injury seems to be related to the physicochemical properties of the antigen-antibody complexes such as the molecular size, the antigen-antibody ratio and the nature of the involved antigen (Cochrane & Dixon, 1976). These characteristics determine both the rate of clearance of the circulating IC by phagocytotic cells and the ability of the IC to penetrate into the blood vessel walls and to incite a local inflammatory reaction.

In none of the biopsies from lesional skin of our patients did immunofluorescence reveal the presence of deposited immunoglobulins in or around vessel walls. Positive IF findings were obtained only with complement C3 and fibrin. This is in accordance with findings of Braverman & Yen (1975) and Sams *et al.* (1975), suggesting that deposited IC, after triggering local inflammatory processes, are rapidly removed by phagocytosis. Thus, it is not surprising that in biopsies of lesional skin only the remnants of the reaction such as tissue bound C3 fragments and precipitated fibrin can be observed.

After histamine injection into unaffected skin of patients with allergic vasculitis, it is possible to study the very early steps in the reaction sequence of IC vasculitis (Braverman & Yen, 1975; Sams *et*

al., 1976; Gower *et al.*, 1976). In 3 of our 4 cases, IgM localized in and around dermal vessels could be detected by IF.

Until now routine electron microscopy has only been able to suggest the presence of IC on the basis of nonspecific amorphous electron dense material in the surroundings of vessels. We have applied the technique of immunoelectronmicroscopy (IEM) which is able to demonstrate specifically immunoglobulins and complement at the ultrastructural level, in the form of distinct peroxidase positive granules. In all of our cases, complement C₃ has been localized precisely between the cellular components and the different layers of the basal lamina of small postcapillary venules. The observation that only postcapillary venules are affected is in accordance with results of Braverman & Yen (1975) obtained with routine electron microscopy. In all examined cases, IgG was demonstrated by IEM although IF findings were negative. This higher sensitivity of IEM as compared to IF has already been reported by Holubar, Konrad & Stingl (1977) on the basis of findings in herpes gestationis.

The specificity of the peroxidase-antiperoxidase multistep technique which has already been pointed out in a number of reports (reviewed by Maciejewski, Wolff & Schmoekel, 1978) is illustrated by the positive staining reaction with membrane bound immunoglobulin of some lymphocytes present in the tissue sections, which are thus characterized as B-lymphocytes (Fig. 5). The C₃ positive material which could occasionally be demonstrated on the surface of macrophages (Fig. 6) consists most probably of antigen-antibody complexes covered by complement components. These opsonized particles become attached to the macrophage membrane by immune adherence, e.g. interaction with C₃b receptors present on the macrophage surface. Since C₃b receptors are also present on B-lymphocytes, but not on T-lymphocytes (Bianco, 1977), the cell which is in contact with the macrophage in Fig. 6 is probably a T-lymphocyte. The fate of antigen-antibody complexes trapped by immune adherence depends on the composition of the complex. If IgG is involved, the macrophage is activated by means of additional surface interaction with Fc receptors of the macrophage and the IC is rapidly ingested by phagocytosis. Evidence of mouse experiments suggests that if the complexes bound by immune adherence are made up with IgM instead of IgG no such interiorization occurs. The complex remains on the surface of the macrophage. Since IgM constitutes the early humoral immune response it has been speculated (Bianco & Nussenzweig, 1977) that this phenomenon serves to concentrate the antigen for further immune processing on the surface of cells bearing C₃b receptors, such as macrophages and B-lymphocytes. In view of this hypothesis, and our present knowledge of helper and suppressor functions of T-lymphocytes in the immune response, it is tempting to speculate on the physiological significance of the macrophage-T-lymphocyte interaction which can be seen in Fig. 6.

The ultrastructural localization of IC has already been demonstrated in animal models during experimental investigations of the active and passive Arthus reaction (Cochrane & Janoff, 1974; Weber *et al.*, 1975). Among the human vasculitides, erythema elevatum diutinum is considered to be a special form of leukocytoclastic vasculitis. In this disorder, bacterial antigens can be used to provoke typical histomorphological changes (Cream, Levene & Calnan, 1971; Katz *et al.*, 1977). Recently, we examined immunoelectronmicroscopically the positive reaction which developed after the injection of streptococcal antigens 24 h after antigen injection in a patient with erythema elevatum diutinum (Wolff *et al.*, 1978). The ultrastructural examination of this material revealed the full sequence of immunological and morphological events. The localization of peroxidase deposits was identical with that found in the present study and, because the material was obtained 24 h after the injection of antigen, later events could also be observed. Together with these results, the present study provides further evidence supporting the current concept of the pathogenesis of human leukocytoclastic vasculitis (Copeman, 1970; Sams *et al.*, 1976).

The present concept of the mechanism involved in the development of IC vasculitis is summarized in Fig. 8. Evidence mainly derived from experimental animal models suggests that circulating IC are not pathogenic by themselves, but require additional pathogenic mechanisms to cause vascular damage: Soluble circulating IC or free antigens react with basophils and cause the release of platelet activating factor (PAF) (Henson & Pincard, 1977). In addition IC themselves directly

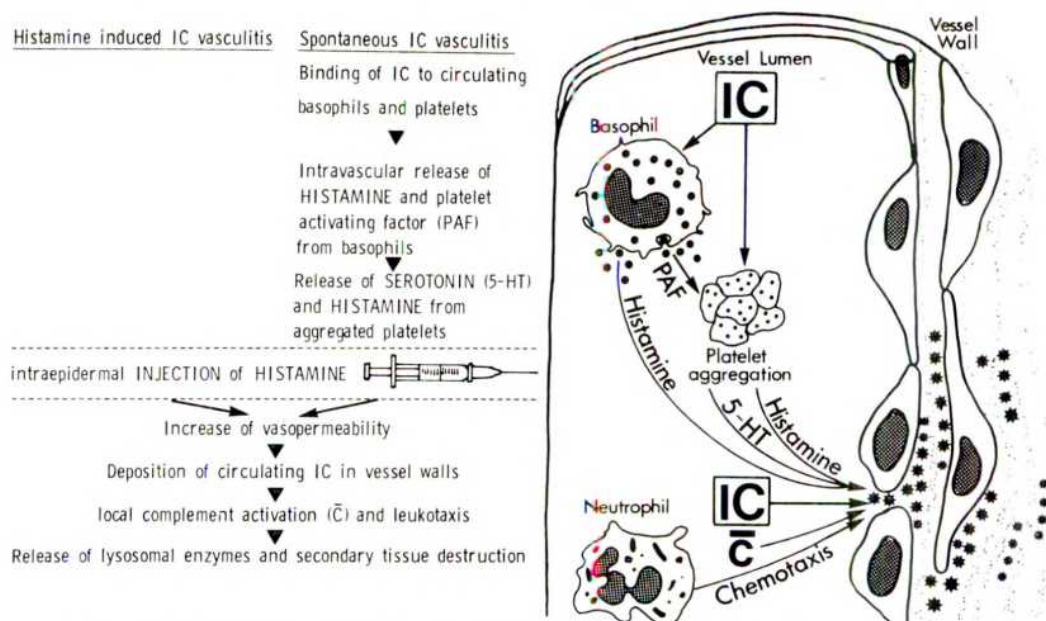


FIGURE 8. Schematic drawing of the sequence of events in spontaneous and in histamine induced immune complex (IC) vasculitis.

cause platelet aggregation by multilocal reaction with Fc- and C₃-receptors present in the platelet membrane. Discharge of histamine from basophils and the release of vasoactive 5-hydroxytryptamine (serotonin) and histamine from aggregated platelets result in a local increase of vasopermeability, which seems to be the prerequisite for the deposition of circulating IC in the vessel walls (Cochrane & Dixon, 1976). Thus the intradermal injection of histamine into unaffected skin in patients with allergic vasculitis replaces the first step in the pathogenesis of IC vasculitis by producing experimentally a local increase in vasopermeability.

Due to the increase in permeability, circulating IC become lodged within the vessel walls and in their vicinity both in experimental and in spontaneous IC vasculitis. The further reaction sequence is also identical: local complement activation triggers a potent amplification system resulting in chemotactic attraction of PMNs, fibrin deposition, leukocytoclasia, secondary vascular damage and inflammatory tissue destruction.

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REFERENCES

- BIANCO, C. (1977) Plasma membrane receptors for complement. In: *Contemporary Topics in Molecular Immunology* (Ed. by R.R.Porter and G.L.Ada), Vol. 6, p. 69. Plenum Press, New York.

- BIANCO, C. & NUSSENZWEIG, V. (1977) Complement receptors. In: *Biological Amplification Systems in Immunology* (Ed. by N.K.Day and R.A.Good), p. 145. Plenum Medical Book Company, New York.
- BRAUN-FALCO, O., MÄNNEL, CH. & SCHERER, R. (1977) Nachweis von zirkulierenden, löslichen Immunkomplexen im Serum von Psoriasispatienten mit dem ^{125}I -C1q-Ablenkungstest. *Hautarzt*, **28**, 658.
- BRAVERMAN, I.M. & YEN, A. (1975) Demonstration of immune complexes in spontaneous and histamine-induced lesions and in normal skin of patients with leukocytoclastic angitis. *Journal of Investigative Dermatology*, **64**, 105.
- COCHRANE, C.G. & DIXON, F.J. (1976) Antigen-antibody complex induced disease. In: *Textbook of Immunopathology* (Ed. by P.A.Miescher and H.J.Müller-Eberhard), 2nd edn, Vol. 1, p. 137. Grune & Stratton, New York.
- COCHRANE, C.G. & JANOFF, A. (1974) The Arthus reaction: a model of neutrophil and complement-mediated injury. In: *The Inflammatory Process* (Ed. by B.W.Zweifach, L.Grant, R.T.McCluskey), 2nd edn, p. 85. Academic Press, New York.
- COPEMAN, P.W.M. (1970) Investigations into the pathogenesis of acute cutaneous angitis. *British Journal of Dermatology*, **82**, Suppl. 5, 51.
- CREAM, J.J., BRYCESON, A.D.M. & RYDER, G. (1971) Disappearance of immunoglobulin and complement from the Arthus reaction and its relevance to the studies of vasculitis in man. *British Journal of Dermatology*, **84**, 106.
- CREAM, J.J., LEVENE, G.M. & CALNAN, C.D. (1971) Erythema elevatum diutinum: an unusual reaction to streptococcal antigen and response to dapsone. *British Journal of Dermatology*, **84**, 393.
- GIANETTI, A., SERRI, F., & BERNASCONI, C. (1976) Immunofluorescent studies of the skin in mixed cryoglobulinemia and Schönlein-Henoch purpura. *Acta dermato-venereologica*, **56**, 211.
- GOWER, R.G., SAMS, W.M., JR, THORNE, G. & CLAMAN, H.N. (1976) Immune complex deposition in leukocytoclastic vasculitis. *Journal of Investigative Dermatology*, **66**, 271A.
- HENSON, P.M. & PINCKARD, R.N. (1977) Platelet activating factor (PAF). A possible direct mediator of anaphylaxis in the rabbit and a trigger for the vascular deposition of circulating immune complexes. *Monographs in Allergy*, **12**, 13.
- HOLUBAR, K., KONRAD, K. & STINGL, G. (1977) Detection by immunoelectron microscopy of immunoglobulin G deposits in skin of immunofluorescence negative herpes gestationis (Brief Communication). *British Journal of Dermatology*, **96**, 569.
- HOLUBAR, K., WOLFF, K., KONRAD, K. & BEUTNER, E.H. (1975) Ultrastructural localization of immunoglobulins in bullous pemphigoid skin. Employment of a new peroxidase-antiperoxidase multistep method. *Journal of Investigative Dermatology*, **64**, 220.
- JONES, J.V., CUMMING, R.H., ASPLIN, C.M., HARMANN, R.R.M. & TRIBE, C.R. (1976) Necrotizing vasculitis: a circulating immune complex producing inflammatory skin lesions. *British Journal of Dermatology*, **94**, 123.
- KATZ, S.I., GALLIN, J.I., HERTZ, K.C., FAUCI, A.S. & LAWLEY, T.J. (1977) Erythema elevatum diutinum: Skin and systemic manifestations, immunologic studies, and successful treatment with dapsone. *Medicine*, **56**, 443.
- MACIEJEWSKI, W., WOLFF, H.H. & SCHMOECKEL, CH. (1978) Immunelektronenmikroskopie in der Dermatologie. *Hautarzt*, in press.
- PARISH, W.E. & RHODES, E.L. (1967) Bacterial antigens and aggregated gamma globulin in the lesions of nodular vasculitis. *British Journal of Dermatology*, **79**, 131.
- PARISH, W.E. (1973) Cutaneous vasculitis. In: *Immunopathology of the Skin: Labelled Antibodies Studies* (Ed. by E.H.Beutner, T.P.Chorzelski, S.F.Bean, R.E.Jordon), p. 153. Dowden, Hutchinson and Ross, Stroudsburg.
- PERROT, H., LEUNG, T.K., LEUNG, J., SCHMITT, D. & THIVOLETT, J. (1971) Etude ultrastrurale des lesions vasculaires dermiques du trisyndrome de Gougerot (vasculite leucocytoclasique). *Archiv für Dermatologische Forschung*, **241**, 44.
- RUITER, M. & MOLENAAR, J. (1970) Ultrastructural changes in arteriolitis (vasculitis) allergica cutis superficialis. *British Journal of Dermatology*, **83**, 14.
- SAMS, W.M.JR., CLAMAN, H.N., KOHLER, P.F., MCINTOSH, R.M., SMALL, P. & MASS, M.F. (1975) Human necrotizing vasculitis: immunoglobulins and complement in vessel walls of cutaneous lesions and normal skin. *Journal of Investigative Dermatology*, **64**, 441.
- SAMS, W.M.JR., THORNE, E.G., SMALL, P., MASS, M.F., MCINTOSH, R.M. & STANFORD, R.E. (1976) Leukocytoclastic vasculitis (Review article). *Archives of Dermatology*, **112**, 219.
- SCHROETER, A.L., COPEMAN, P.W.M., JORDON, R.E., SAMS, W.M., JR & WINKELMANN, R.K. (1977) Immunofluorescence of cutaneous vasculitis associated with systemic disease. *Archives of Dermatology*, **104**, 254.

- SOBEL, A.T., BOKISCH, V.A. & MÜLLER-EBERHARD, H.J. (1975) C1q deviation test for the detection of immune complexes, aggregates of IgG and bacterial products in human serum. *Journal of Experimental Medicine*, **142**, 139.
- WEBER, K., UEKI, H., WOLFF, H.H. & BRAUN-FALCO, O. (1975) Ultrastructural evidence for lack of tissue damage in a local immune complex reaction. A study of a mild passive Arthus reaction. *Virchows Archives of Biological Cell Pathology*, **18**, 213.
- WOLFF, H.H., SCHERER, R., MACIEJEWSKI, W. & BRAUN-FALCO, O. (1978) Erythema elevatum diutinum. II. Immunelektronenmikroskopische Untersuchung der leukocytoklastischen Vasculitis in einer Intrakutanreaktion mit Streptokokkenantigen. *Archives for Dermatological Research*, in press.

Immunological aspects of psoriasis

III. Fc- γ -RECEPTOR BEARING MONONUCLEAR CELLS IN PERIPHERAL BLOOD

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SUMMARY

An anti-IgG activity has previously been reported at the cellular level in patients with psoriasis. This activity was demonstrated by the so-called 'rheumatoid' rosette test. In the present work the nature of 'rheumatoid' rosette-forming cells was studied in comparison with other EA rosette techniques. The use of purified cell populations showed that the lymphocytes participating in the 'rheumatoid' rosette phenomenon were lacking conventional T and B cell membrane markers, and were thus referred to as null cells. Such mononuclear cells bearing a receptor for the Fc part of IgG were able to act as killer cells to IgG-coated target cells. The cytotoxic activity was mainly restricted to a small proportion of lymphocytes forming 'rheumatoid' rosettes which had a high avidity for EA complexes. Such cytotoxicity could contribute to the aetiology of lesions in psoriasis.

Several immunological disturbances are now recognized in psoriasis (Guilhou, Clot & Meynadier, 1978). One such disturbance is an anti-IgG activity on psoriatic peripheral blood lymphocytes first described by Rimbaud *et al.* (1973), using the so-called 'rheumatoid'-rosette test (Bach, Delrieu & Delbarre, 1970). It has been demonstrated that the 'rheumatoid' rosette test is similar to the EA-rosette test (Clot *et al.*, 1974). This phenomenon should be re-examined following the recent reports of mononuclear cells bearing receptors for the Fc fragment of IgG (Fc- γ). These cells can be detected by rosette techniques by mixing mononuclear cells and erythrocyte-antibody (EA) complexes.

Moreover it has been reported that Fc- γ -receptor bearing cells can act as killer cells (K cells) in cytotoxic reaction referred to as antibody dependent cell cytotoxicity (ADCC).

The presence of immunoglobulins and numerous auto-antibodies in psoriatic epidermis (review in Guilhou *et al.*, 1978) and the skin infiltration by mononuclear cells (Braun-Falco & Christophers, 1974), (despite the absence of direct signs of cytotoxicity in the psoriatic epidermis) led us to study the nature and the lytic activity of Fc- γ -receptor bearing cells detected in psoriatic peripheral blood by several EA-rosette techniques and by an ADCC assay. Such mononuclear cells could participate in the skin lesions by coating to IgG antibodies bound to epidermal cells.

MATERIAL AND METHODS

Lymphocyte suspension

Lymphocytes were isolated, from the peripheral blood of patients with psoriasis and of healthy human donors, by rapid filtration on a nylon column to remove monocytes before centrifugation on Ficoll-Hypaque density gradient (400 g, 30 min., 20°C).

EA-rosette forming mononuclear cells (EA-RFC)

Three techniques using erythrocyte (E)-antibody (A) complexes were used. The so-called 'rheumatoid' rosette test (R-RFC), according to a method previously reported (Rimbaud *et al.*, 1973) with fresh, human group O, rhesus D negative erythrocytes coated with small amounts (1:4000) of inactivated rabbit anti-human erythrocyte IgG (Pr. A. Eycuém, Institut Pasteur, Paris). Rosettes were formed after a short centrifugation (5 min, 200 g, 4°C).

The two other EA rosette techniques were performed according to Brochier, Samarut & Revillard (1975): chicken red blood cells (CRBC) were coated with rabbit anti-CRBC IgG either at subagglutinating concentration (EA-Ch-RFC) or at 1:4000 antiserum concentration ('high-avidity' EA-Ch-RFC; (Cordier *et al.*, 1976)).

Depletion of rosette forming cells (RFC)

In order to obtain purified lymphocyte suspensions two methods were used. Adherent cells (monocytes and B-lymphocytes) were removed by slow filtration on nylon fibres (Brown & Greaves, 1974). Separation of cells after rosette formation, as previously described (Clot, Massip & Mathieu, 1975) for E-rosettes, was adapted for each type of rosettes. The presence of T and B lymphocytes in purified cell suspensions was assessed by classical T and B cell membrane markers (Clot *et al.*, 1978).

Antibody dependent cell cytotoxicity assay (ADCC)

ADCC was measured according to a method already reported (Cordier & Clot, 1976). Briefly, ⁵¹Cr-labelled CRBC were mixed with anti-CRBC serum and peripheral blood lymphocytes from non-immune donors. Antiserum dilution (5×10^{-4}), incubation time (5 h) and effector to target cell ratios (40:1, 20:1, 10:1 and 5:1) were selected so that variations in actual numbers of effector cells resulted in predictable changes of the cytotoxic indexes. Results were expressed as cytotoxic indexes as defined by Cerottini & Brunner (1974).

RESULTS

Technical conditions for EA-RFC

It has been demonstrated previously (Sany *et al.*, 1975) that R-RFC detected 0.5–1% of lymphocytes in normal peripheral blood if the usual technical conditions were used (5 min centrifugation, 200 g, 4°C). However, an overnight incubation at 4°C significantly increased the number of R-RFC (up to 9–12% in normal peripheral blood).

EACH-RFC were formed after 5 min centrifugation (200 g, 4°C) by 10–15% of peripheral blood lymphocytes from healthy human donors (Brochier *et al.*, 1975). A longer incubation time did not significantly increase EACH-RFC. Conversely, 'high-avidity' EACH-RFC detected 0.2–2.5% of normal lymphocytes when a 5 min centrifugation was used (Cordier *et al.*, 1976). As for R-RFC, a longer incubation period increased the RFC numbers.

Lymphocyte sub-population involved in R-RFC

In order to study the lymphocyte subset involved in 'rheumatoid' rosette formation, different lymphocyte suspensions were prepared and some lymphocyte membrane markers (E-rosette, surface

TABLE 1. R-RFC before and after depletion of adherent cells on nylon columns

	E-RFC*		SIg*		EA-Ch RFC*		R-RFC*	
	Before	After	Before	After	Before	After	Before	After
Normal Donors (n = 12)	70.8 ± 1.2	75.2 ± 0.9	14.2 ± 0.6	1.1 × 0.2	12.5 ± 0.5	8.9 ± 0.8	0.6 ± 0.1	2.4 ± 0.4
Psoriasis (n = 15)	59.1 ± 2.1	63.5 ± 1.9	15.1 ± 0.7	0.9 ± 0.1	12.9 ± 0.6	9.0 ± 0.3	2.6 ± 0.2	4.9 ± 0.3

* E-RFC, Surface immunoglobulins, EACh-RFC and R-RFC numbers expressed as mean percentage ± s.e.

immunoglobulins) were used to determine the purity of each cell suspension. EACh-RFC were studied simultaneously in comparison with R-RFC.

The depletion of adherent cells on nylon columns (Table 1) removed most B lymphocytes (Brown *et al.*, 1974), whereas the proportion of T cells was increased. The percentage of EACh-RFC was slightly decreased demonstrating the participation of adherent cells (B cells) in their formation (Samarut, Brochier & Revillard, 1976). Conversely, the percentage of R-RFC from both normal donors and selected psoriatic patients (having high percentage of R-RFC in peripheral blood) was increased after removal of adherent cells.

Starting from peripheral blood lymphocytes, the centrifugation on Ficoll gradient of E-RFC provided an E-RFC enriched cell suspension (E^+) in the pellet, and a E-RFC-depleted cell suspension (E^-) at the interface (Table 2). In such purified populations, R-RFC were recovered in the E-RFC depleted suspension (E^-). If this last population containing mainly B cells (86.4%) was filtered through nylon columns, the adherent cells were removed and only 1.6% of cells bearing surface immunoglobulins were detected after filtration. In this experiment, the percentage of R-RFC was

TABLE 2. R-RFC in various cell populations purified by centrifugation of E-RFC from psoriatic peripheral blood lymphocytes. Results of 7 experiments

	E-RFC	SIg	EACh-RFC	R-RFC
Unseparated*	61.3 ± 2.1*	15.1 ± 0.8	12.6 ± 0.6	3.1 ± 0.3
E^+ †	88.5 ± 1.5	3.7 ± 0.9	3.1 ± 1.1	0.1 ± 0.06
E^- ‡	4.3 ± 1.2	86.4 ± 2.9	41.5 ± 2.0	12.9 ± 1.9
E^- -nylon§	n.d.	1.6 ± 0.1	53.1 ± 3.1	29.1 ± 2.2
E^- -RR-¶	n.d.	85.9 ± 1.6	52.9 ± 2.8	2.0 ± 1.1

*Results expressed as mean percentage ± s.e.; n.d. = not done.

† E^+ = cells from the pellet of centrifugation on Ficoll gradient after mixing peripheral blood lymphocytes and sheep red blood cells (E-RFC-enriched suspension).

‡ E^- = cells collected at the interface after centrifugation as above indicated (E-RFC-depleted suspension).

§ E^- -nylon = E-RFC-depleted suspension after passing through a nylon column to remove adherent cells.

¶ E^- -RR- = E-RFC-depleted suspension mixed with EA complexes to form rheumatoid rosettes; the cells studied were collected at the interface after centrifugation on Ficoll gradient.

TABLE 3. Antibody dependent cell cytotoxicity (ADCC) in normal and psoriatic peripheral blood

No. of experiments	Effector: target cell ratios			
	40:1 (10)	20:1 (8)	10:1 (10)	5:1 (8)
Normal donors	52.9 ± 3.2*	48.6 ± 6.0	36.5 ± 7.6	28.4 ± 6.9
Psoriatic patients	65.2 ± 2.7	49.7 ± 3.4	44.6 ± 3.3	30.5 ± 4.6
Student's 't'-test on paired value†	$P < 0.01$	ns	ns	ns

* Results expressed as cytotoxic index (mean ± s.e.); ns = not significant.

† The Student's *t*-test was carried out on each pair, psoriatic patients-normal donors, studied in the same ADCC experiment.

clearly increased (29.1%) in a cell suspension thus lacking T cells and B cells. In the same way, if R-RFC were removed from E-RFC-depleted suspension (E^-RR^-) by the centrifugation of rosettes Ficoll gradient, the B-cell percentage was not modified (85.9% vs 86.4%).

ADCC of peripheral blood lymphocytes from normal and psoriatic subjects

The results are presented in Table 3. Because of differences in lymphocytes donors and target cells, the cytotoxic activity of the lymphocytes was not identical from one experiment to the next. To compensate for this variation, each ADCC experiment included a psoriatic patient and a normal donor; the comparisons were made by Student's *t*-test on each paired value.

No statistical difference appeared between normal donor and psoriatic patient lytic activities, except for the higher effector: target cell ratio (40:1).

ADCC after depletion of EA-RFC

The data reported in Table 4 were obtained from selected psoriatic patients having high R-RFC percentages. By using the centrifugation of RFC on Ficoll gradient, three cell suspensions, collected at the interface, were prepared and ADCC experiments were made on these suspensions in a similar test. After depletion of EACh-RFC, the remaining cells were unable to mediate ADCC. The following

TABLE 4. ADCC after depletion of EA-RFC from psoriatic blood lymphocytes. Results of 6 experiments

Lymphocyte suspensions	EACH-RFC	'High avidity' EACH-RFC	R-RFC	Cytotoxic† index
Unseparated	12.7 ± 0.6*	1.5 ± 0.2	2.6 ± 0.3	63.8 ± 2.1
After depletion of EACH-RFC	0.6 ± 0.4	n.d.	0	2.1 ± 1.8
After depletion of 'high avidity' EACH-RFC	11.8 ± 0.7	0.2 ± 0.1	0.1 ± 0.1	3.4 ± 1.2
After depletion of R-RFC	11.9 ± 0.9	n.d.	0.1 ± 0.08	1.9 ± 1.0

* Results expressed as mean ± s.e.; n.d. = not done.

† Cytotoxic index measured at a 40:1 effector to target cell ratio.

experiments demonstrated that all cells forming EA rosettes could not act as effector cells in ADCC. Effectively, the cell suspensions depleted of 'high-avidity' EAC_H-RFC or of R-RFC were almost completely depleted of effector cells mediating ADCC. In contrast, the numbers of EAC_H-RFC were very slightly modified by the depletion of R-RFC (and of 'high avidity' EAC_H-RFC). The lytic activity was thus limited to a small sub-population of Fc-receptor bearing cells.

DISCUSSION

The data reported here can be summarized as follows:

- (a) The 'rheumatoid' rosette-forming cells have a high avidity to EA complexes.
- (b) They are null-cells without T or B cell markers.
- (c) They are able to mediate antibody-dependent cell cytotoxicity (ADCC).
- (d) Finally, the so-called 'rheumatoid' rosettes are EA-rosettes.

'High avidity' EAC_H-RFC (Cordier *et al.*, 1976) and R-RFC seem very similar: low amounts of sensitizing antibodies, low percentages in peripheral blood, and lymphocytes defined as mainly not-T and not B cells (null-cells). However, the nature of the lymphocyte population involved in the EA-rosette formation remains a matter of controversy. The test appears to depend on the source of erythrocytes and on the amount of sensitizing antibodies. The EA-RFC have been identified either as B cells (Brain & Marston, 1973), or as null-cells (Froland, Wisloff & Michaelsen, 1974), or as T, B and null-cells (Samarut *et al.*, 1976), or as T cells (Ferrarini *et al.*, 1975). The presence in psoriatic blood of high proportions of R-RFC defined as null-cells could have relevance to the report of an increased null-cell percentage in peripheral blood from psoriatics (Clot *et al.*, 1978).

Fc- γ -receptor bearing cells can act as killer cells in the ADCC test. In such an *in vitro* reaction, target cells coated with specific IgG antibodies can be destroyed by the addition of effector cells from non-immune donors (Perlmann & Holm, 1968). The Fc part of IgG binds to the Fc- γ -receptors of the effector cells (Larsson, Perlmann & Natvig, 1973; Mac Lennan, 1972). In man, several cells have been shown to mediate ADCC, but the cytotoxicity test used in this work was been demonstrated to require effector cells lacking conventional B or T cell membrane markers (Calder *et al.*, 1974; Wisloff, Froland & Michaelsen, 1974; Cordier *et al.*, 1976). Our present data confirm these findings: the effector cell capacity in ADCC was restricted to a small subpopulation of cells detected by 'high avidity' EAC_H-RFC or by R-RFC. In psoriasis, ADCC was nearly similar to that in normal donors and a relationship was difficult to make between the R-RFC percentage in blood and the cytotoxic activity (unpublished data); this could be due to the low proportions of R-RFC.

The report of an anti-IgG activity on some psoriatic peripheral blood lymphocytes (Rimbaud *et al.*, 1973) can be thus considered to be due to the presence of Fc- γ -receptor cells with a high avidity for IgG and not to the presence of rheumatoid factor on lymphocytes. Some psoriatic patients (about 40%) have higher percentages of such cells than normal human donors. At the present time, no evidence supports a possible *in vivo* relationship between Fc- γ -receptor lymphocytes and circulating immune complexes such as rheumatoid factor (Diaz-Jouanen, Bankhurst & Williams, 1976; Sany *et al.*, 1976). However, such an interaction has been reported *in vitro* (Cordier, Samarut & Revillard, 1978), and an anti-IgG activity was described both in psoriatic serum (Guilhou *et al.*, 1976) and in psoriatic skin (Krogh & Tonder, 1972). In addition the exact role of such a lymphocyte subpopulation has to be considered in psoriasis since Fc- γ -receptor on T cells is a marker of suppressor cells (Fridman *et al.*, 1975) which could be related to the pathogenesis of the disease.

REFERENCES

- BACH, J.F., DELRIEU, F. & DELBARRE, F. (1970) The rheumatoid rosette: a diagnostic test unifying seropositive and seronegative rheumatoid arthritis. *American Journal of Medicine*, **49**, 213.

- BRAIN, P. & MARSTON, R.H. (1973) Rosette formation by human T and B lymphocytes. *European Journal of Immunology*, **3**, 6.
- BRAUN-FALCO, O. & CHRISTOPHER, E. (1974) Structural aspects of initial psoriatic lesions. *Archiv für Dermatologische Forschung*, **251**, 95.
- BROCHIER, J., SAMARUT, C. & REVILLARD, J.P. (1975) A rosette technique for identification of human mononuclear cells bearing Fc receptors. *Biomedicine Express*, **23**, 206.
- BROWN, G. & GREAVES, M.F. (1974) Cell surface markers for human T and B lymphocytes. *European Journal of Immunology*, **4**, 302.
- CALDER, E.A., URBANIAK, S.J., PENHALE, W.J. & IRVINE, W.J. (1974) Characterization of human lymphoid cell-mediated antibody-dependent cytotoxicity (LDAC). *Clinical and Experimental Immunology*, **18**, 579.
- CEROTTINI, J.C. & BRUNNER, K.T. (1974) Cell mediated cytotoxicity, allograft rejection and tumor immunity. *Advances in Immunology*, **18**, 67.
- CLOT, J., SANY, J., MASSIP, H. & SERRE, H. (1974) Le phénomène des rosettes rhumatoïdes: note préliminaire sur les sous-populations lymphocytaires impliquées. *Nouvelle Presse Médicale*, **3**, 2414.
- CLOT, J., MASSIP, H. & MATHIEU, O. (1975) *In vitro* studies on human B and T cell purified populations. Stimulation by mitogens and allogeneic cells and quantitative binding of phytomitogens. *Immunology*, **29**, 445.
- CLOT, J., DARDENNE, M., BROCHIER, J., ANDARY, M. & GUILHOU, J.J. (1978) Evaluation of lymphocyte subpopulations and T cell functions in psoriasis. *Clinical Immunology and Immunopathology*, **9**, 389.
- CORDIER, G., SAMARUT, C., BROCHIER, J. & REVILLARD, J.P. (1976) Antibody dependent cell cytotoxicity (ADCC). Characterization of 'Killer' cells in human lymphoid organs. *Scandinavian Journal of Immunology*, **5**, 233.
- CORDIER, G. & CLOT, J. (1976) Microméthode de cytotoxicité à médiation cellulaire pour la mise en évidence des cellules K, in Techniques of separation and characterization of human lymphocytes INSERM, **57**, 219.
- CORDIER, G., SAMARUT, C. & REVILLARD, J.P. (1978) Changes of Fc- γ -receptor related properties induced by interaction of human lymphocytes with insoluble immune complexes. *Journal of Immunology*, **119**, 1943.
- DIAZ-JOUANEN, E., BANKHURST, A.D. & WILLIAMS, F.C. (1976) Antibody-mediated lymphocytotoxicity in rheumatoid arthritis and systemic lupus erythematosus. *Arthritis and Rheumatism*, **19**, 133.
- FERRARINI, M., MORETTA, L., ABRILE, R. & DURANTE, M.L. (1975) Receptors for IgG molecules on human lymphocytes forming spontaneous rosettes with sheep red cells. *European Journal of Immunology*, **5**, 70.
- FRIDMAN, W.H., NEAUPORT-SAUTES, C., GUIMEZANES, A. & GASLER, R.H. (1975) Relation between Fc receptors of activated T cells, immunoglobulin binding factor (IBF) and suppression of *in vitro* antibody synthesis. In: *Membrane Receptors of Lymphocytes* (Ed. by M. Seligman, J.L. Pred'homme and F.M. Kourilsky), p. 217. North Holland, Amsterdam.
- FROLAND, S.S., WISLOFF, F. & MICHAELSEN, T.E. (1974) Human lymphocytes with receptors for IgG. A population of cells distinct from T and B lymphocytes. *International Archives of Allergy*, **47**, 124.
- GUILHOU, J.J., CLOT, J., MEYNADIER, J., & LAPINSKI, H. (1976) Immunological aspects of psoriasis. I. Immunoglobulins and anti-IgG factors. *British Journal of Dermatology*, **94**, 501.
- GUILHOU, J.J., CLOT, J., MEYNADIER, J. (1978) New concepts in psoriasis pathogenesis. *British Journal of Dermatology*, **98**, 585.
- KROGH, H.K. & TONDER, O. (1972) Immunoglobulins and anti-immunoglobulin factors in psoriatic lesions. *Clinical and Experimental Immunology*, **10**, 623.
- LARSSON, A., PERLMANN, P. & NATVIG, J.B. (1973) Cytotoxicity of human lymphocytes induced by rabbit antibodies to chicken erythrocytes. Inhibition by normal IgG and by human myeloma proteins of different IgG sub classes. *Immunology*, **25**, 675.
- MAC LENNAN, I.C.M. (1972) Antibody in the induction and inhibition of lymphocyte cytotoxicity. *Transplantation Review*, **13**, 67.
- PERLMANN, P. & HOLM, G. (1968) Studies on the mechanism of lymphocyte cytotoxicity. In: *Mechanisms of Inflammation Induced by Immune Reactions* (Ed. by P. Miescher and P. Grabar), p. 325. Schabe and Co., Basel.
- RIMBAUD, P., MEYNADIER, J., GUILHOU, J.J. & CLOT, J. (1973) Anti-IgG activity on peripheral blood lymphocytes in psoriasis. *Archives of Dermatology*, **108**, 371.
- SAMARUT, C., BROCHIER, J. & REVILLARD, J.P. (1976) Distribution of cells binding erythrocyte-antibody (EA) complexes in human lymphoid populations. *Scandinavian Journal of Immunology*, **5**, 221.
- SANY, J., CLOT, J., MASSIP, H., CHARMASSEN, E. & SERRE, H. (1975) Sous-populations lymphocytaires impliquées dans le phénomène des rosettes rhumatoïdes. *Revue du Rhumatisme*, **42**, 309.
- SANY, J., CLOT, J., FREITAS, M., CHARMASSEN, E. & SERRE, H. (1976) Cytotoxicité anticorps dépendante des lymphocytes sanguins au cours de la polyarthrite rhumatoïde. *Revue du Rhumatisme*, **43**, 333.
- WISLOFF, F., FROLAND, S.S. & MICHAELSEN, T.E. (1974) Antibody-dependent cytotoxicity mediated by human Fc-receptor-bearing cells lacking markers for B and T lymphocytes. *International Archives of Allergy and Applied Immunology*, **47**, 139.

IgA and C3 complement in the uninvolved skin in dermatitis herpetiformis after gluten withdrawal

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SUMMARY

IgA deposits in the skin in 53 patients with dermatitis herpetiformis (DH) have been studied in relation to treatment. In 19 patients the disorder was controlled by a gluten-free diet (GFD) alone, in 13 patients by dapsone and GFD and in 18 by dapsone alone. In 3 patients the skin disorder became insignificant and required no treatment. Of the patients taking a GFD alone, six had been clear of skin lesions for 7 years, 5 for 3–5 years, and 8 for periods of 6 months–3 years. IgA deposits were found in all patients in an initial biopsy and in a second biopsy after treatment for periods varying from 1 to 7 years. There was no difference in the quantity of IgA, as assessed by the amount of fluorescence, whether patients were controlled with a GFD alone, GFD and dapsone, dapsone alone, or in those in clinical remission. The C3 component of complement was present in the skin in 3 of the 19 patients (16%) controlled by a GFD alone, 6 of the 13 patients (46%) of those controlled by a GFD and dapsone, and in 12 of 18 (66%) of the patients taking dapsone alone, and in one of the patients in clinical remission.

The presence of IgA in the uninvolved skin of patients with dermatitis herpetiformis (DH) is a characteristic feature of the disease (Jablonska *et al.*, 1973; Fry & Seah, 1974; Seah & Fry, 1975). The significance of the IgA in the uninvolved skin has not been satisfactorily explained. It is possibly related to the abnormality of the small intestine (gluten sensitive enteropathy) which occurs in DH, but IgA is not found in the skin of patients with coeliac disease (Seah *et al.*, 1972). A recent study (Harrington & Read, 1977) has reported that in patients with DH who had taken a gluten free diet (GFD) for 6 months, there was a decrease in the concentration of IgA in the uninvolved skin, and in 2 of 10 patients the IgA was no longer present. Harrington & Read (1977) stated that a decrease in the IgA in the skin was one of the objective results of GFD in DH. If this were correct it might be assumed that the longer the patients had taken a GFD and had their rash controlled by it, then the less IgA would be found in the skin, and it might eventually disappear. We have studied patients with DH who have been on a GFD for periods up to 9 years and whose rash is controlled with this diet and report our results.

PATIENTS AND METHODS

Fifty-three patients (23 females and 31 males), whose ages ranged from 21 to 71 years, were studied. The patients were divided into four groups depending on their treatment.

Group 1. Nineteen patients whose skin eruption was controlled by a GFD alone. Six patients had been clear of the eruption for 7 years or longer, 5 patients for between 3 and 5 years and 8 patients for between 6 months and 3 years.

Group 2. Thirteen patients who were taking a GFD, but who also required dapsone for control of their eruption. The mean dose of dapsone to control the eruption for this group was 400 mg per week (range 25–1400 mg per week). The duration of the GFD varied from 6 months to 5 years.

Group 3. Eighteen patients who were taking a normal diet and required dapsone to control their eruption. The average dose of dapsone to control the eruption in these 18 patients was 800 mg per week (range 100–2100 mg per week).

Group 4. Three patients, taking a normal diet, who originally required dapsone to control their eruption, but who later were able to dispense with it. One patient had DH for 6 years and originally had required dapsone to control the eruption, but for the past 2 years she had been free of skin lesions and irritation, and had taken no dapsone. The other two patients had DH for 8 and 5 years respectively. Neither required dapsone for the last 3 years, although initially it had been essential to control their eruption. They both had occasional irritation and skin lesions on the elbows although neither considered it severe enough to take dapsone again.

Skin biopsies. All patients had at least 2 skin biopsies from the clinically uninvolved skin, the site of biopsy being the flexor aspect of the forearm or the upper and outer buttock. The first biopsy was carried out at the time of the initial diagnosis or 7 years ago when we began to do this test as a routine for all patients with DH. Thus in Group 1, 6 of our patients were already taking a GFD at the time of this first biopsy. The second biopsy which has been compared to the initial one was carried out in the last year on all the patients in this study. The initial biopsy of one patient and the second biopsy of another patient did not show immunoglobulin deposits. The biopsies were repeated and in each instance immunoglobulins were found.

Methods. Skin biopsies were taken under local anaesthesia using lignocaine 1 or 2% and snap frozen as previously described (Seah *et al.*, 1972) and stored at -70°C . The skin biopsies were processed for immunoglobulin deposits using monospecific fluorescein isothiocyanate (FITC) labelled sheep antihuman IgG, IgM and IgA (Wellcome Reagents) at a dilution where minimal non-specific staining was seen as described by Seah *et al.* (1972). The second biopsies were also stained for complement C3 deposits using rabbit antihuman C3 (B1c/B1a) FITC conjugated antiserum (Dako-patts) where minimal non-specific staining was seen. The slides were examined using a Leitz Dialux microscope with Ploem incident illumination from a 50 W ultra-high pressure mercury lamp. The filter system comprised: Exciter $2 \times \text{KP } 490$; dichroic mirror TK 510; suppression K 515 and edge filter GG 455.

IgA fluorescence was graded: +++ (strong); ++ (moderate); + (weak), representing large, intermediate and small amounts of IgA respectively (Figs 1–3).

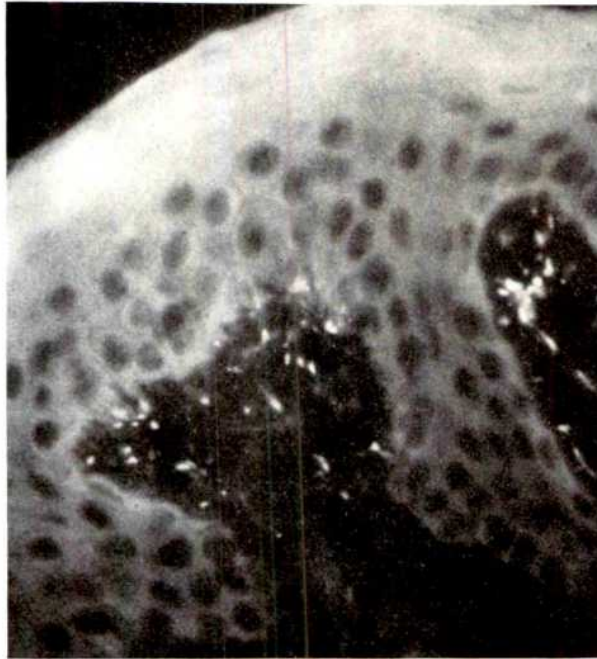


FIGURE 1. Weak immunofluorescence of IgA deposits.

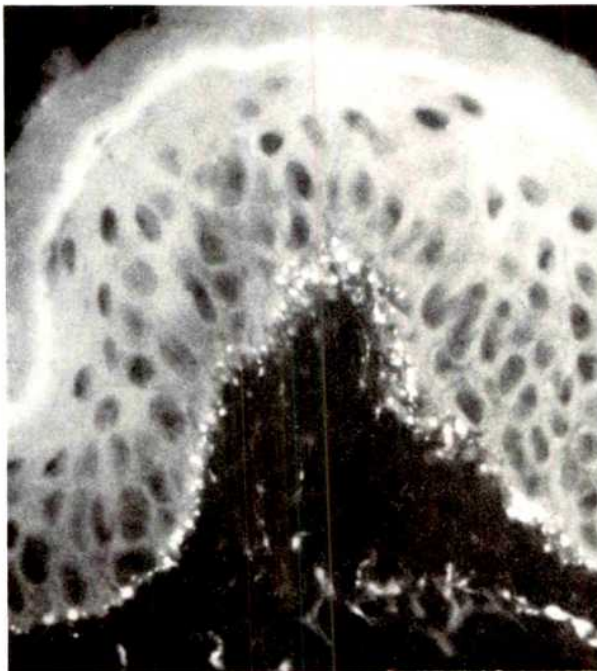


FIGURE 2. Moderate immunofluorescence of IgA deposits.

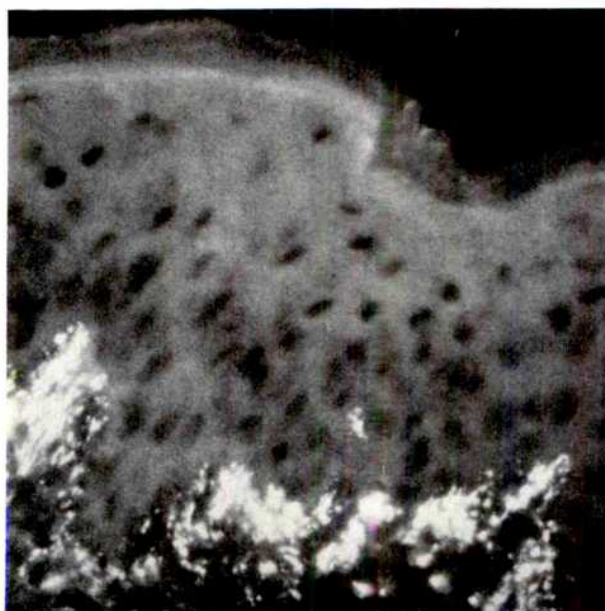


FIGURE 3. Strong immunofluorescence of IgA deposits.

RESULTS

Incidence of immunoglobulin deposits

All 53 patients had IgA deposits in both the first and second biopsies (Table 1). IgM and IgG were found in a small number of patients (Table 1) but there was no difference in the incidence between the first and second biopsies.

Patterns of IgA staining. In the first biopsies the pattern of IgA deposition was papillary in 51 and linear in 2. The 2 biopsies showing linear deposition were both from patients taking dapsone alone. One of the 2 patients continued with dapsone alone and on the second biopsy the pattern of IgA deposition was papillary. In the other patient at the time of the second biopsy the patient was taking a GFD but still required dapsone for control of the rash, and the pattern of IgA deposition remained

TABLE 1. Incidence of immunoglobulins and C3 deposits

	Total	IgA		IgM		IgG		C3
		1st biopsy	2nd biopsy	1st biopsy	2nd biopsy	1st biopsy	2nd biopsy	
GFD only	19	19	19	3	5	1	0	3
GFD & dapsone	13	13	13	5	3	2	1	6
Dapsone only	18	18	18	2	2	0	1	12
No treatment	3	3	3	2	1	1	0	1

linear. One patient receiving dapsone treatment alone had the papillary pattern in the first biopsy which converted to linear in the second biopsy. Thus on the second biopsy the pattern of IgA deposition was papillary in 51 and linear in 2.

Incidence of degrees of IgA staining. In the four groups of patients the incidence of strong, moderate and weak fluorescence were similar, when the first or second biopsies were compared (Table 2). Similarly, when the group of patients taking only a GFD were subdivided into 3 separate groups depending on the length of time the diet had been taken, there was no difference (Table 3).

TABLE 2. Incidence of degrees of IgA staining in relation to treatment

	+++ (strong)		++ (moderate)		+ (weak)	
	1st biopsy	2nd biopsy	1st biopsy	2nd biopsy	1st biopsy	2nd biopsy
GFD only	5	4	10	9	4	6
GFD & dapsone	6	5	5	4	2	4
Dapsone only	5	8	10	7	3	3
No treatment	1	1	2	1	0	1

TABLE 3. Incidence of degrees of IgA staining in relation to length of time of a GFD

	+++ (strong)		++ (moderate)		+ (weak)	
	1st biopsy	2nd biopsy	1st biopsy	2nd biopsy	1st biopsy	2nd biopsy
Diet more than 7 years (6 patients)	1	1	4	2	1	3
Diet 3-5 years (5 patients)	1	0	3	3	1	2
Diet under 3 years (8 patients)	3	3	3	4	2	1

Variation in amount of IgA staining between first and second biopsies. There was a change in the amount of demonstrable IgA in over half of the patients between the first and second biopsy. There was no difference between the groups in the incidence of increase or decrease in amount of IgA staining (Table 4). Thus, patients taking a GFD were just as likely to show an increase in the amount of IgA staining as those taking a normal diet and dapsone.

Incidence of C3 deposits. C3 was found in a number of patients in each of the four groups. The highest incidence, 12 of 18 patients (66%), was in the group being controlled with dapsone alone. Three of 19 (16%) whose eruption was controlled with a GFD alone also had C3 deposits in the skin. In the group taking a GFD and dapsone, C3 was found in 6 of the 13 patients (46%). Of the patients not receiving any treatment at the time of study, C3 was found in one.

TABLE 4. Variation in amount of IgA staining between 1st and 2nd biopsy

	Total	Increase	Decrease	No change
GFD only	19	5	8	6
GFD & dapsone	13	5	5	3
Dapsone only	18	5	3	9
No treatment	3	1	2	0

DISCUSSION

These results show that IgA persists in the uninvolved skin of patients with DH, even though their rash is controlled with a GFD. These findings differ from those of Harrington & Read (1977) who stated that the concentration of IgA in the skin falls after gluten withdrawal. Harrington & Read studied their patients after only 6 months on a GFD, whereas, in the present study, patients had been taking a GFD for periods ranging from 6 months to 9 years. Of the 10 patients studied by Harrington & Read, 4 still required dapsone. If the suggestion of Harrington & Read is correct and the concentration of IgA does fall, and even disappear with a GFD, then it would be expected that the longer the patient had been taking a GFD the more likely it would be for IgA to disappear from the skin. This was not the finding in this study. Six of our patients had been free of all skin lesions for at least 7 years and IgA was still present in the skin of all of them. There appeared to be no difference in the amount of IgA staining in relation to the duration of a GFD which would have been expected if gluten was solely responsible for IgA deposition in the skin. However, the number of patients is relatively small, and possibly a larger series, or study of the patients over longer periods, will show if there is a significant alteration of IgA deposition in relation to a GFD.

Detection of IgA in the skin by immunofluorescence is a technique which is not suitable for accurately quantifying IgA deposition. Visual impressions of brightness of fluorescence can be misleading. Unfortunately IgA deposits in skin cannot be solubilized and titres obtained. This has been done with antinuclear factor, and it has been found that sera giving equal fluorescence can be obtained from titres differing 10 fold; conversely, sera with identical titres may give fluorescent patterns of different visual intensity (Sams, 1974). A further variable is that the subjective impression of the amount of fluorescence may vary from one investigator to another. We have graded fluorescence as strong, moderate and weak, but this can be taken only as a rough guide to the amount of IgA present. It is also known that the deposition of IgA may vary from one part of the skin to another. Jablonska *et al.* (1973) found that if serial sections were made through a biopsy, IgA was present in some of the sections but not in others. Seah & Fry (1975) found no IgA in 2 of 50 patients with DH on a single biopsy, but a repeat biopsy showed IgA to be present. Thus in the study by Harrington & Read, the fact that no IgA was detected in a single biopsy in 2 of their patients cannot be taken to imply that IgA is no longer present in the skin.

In the study by Harrington & Read (1977) the concentration of IgA was determined by the staining pattern, i.e. linear, granular or speckled, in that order. At present the significance of the linear pattern of immunoglobulin deposition is not known, and there is thus no evidence to indicate that this pattern of deposition implies 'more' immunoglobulin than that of the more common pattern beneath the basement membrane. In addition, the presence of both the so-called 'granular' and 'speckled' patterns of IgA deposition in a single biopsy, which we have found to occur, makes it difficult to label a biopsy with this terminology and correlate it to the quantity of IgA.

Apart from the persistence of IgA deposits in the skin after control of the skin lesions by a GFD, C₃ deposits have also been found in a small number (16%) but the incidence does appear to be lower than in those only taking dapsone (66%) and those taking a GFD and dapsone (46%). The presence of C₃ complement means that complement must have been activated at some time and that a disease process is still active. It should be remembered that even in the patients taking dapsone alone there was no clinical skin disease, although it may be argued that dapsone is preventing a stage in the disease process after C₃ activation. It is possible that in the patients taking a GFD, the C₃ is activated by complexes other than those containing gluten, but that no skin lesions will follow unless some other factor such as gluten is present. It is known that immune complexes do persist in patients with DH and coeliac disease controlled by a GFD (Mohammed *et al.*, 1976) and it is possible that these are reticulin complexes which bind to the reticulin fibres and activate complement.

The 3 patients who no longer required dapsone for control of their eruption and who had never taken a GFD are an interesting small group. These patients were symptom-free except for the occasional 'spot' which certainly did not require dapsone. These patients are a minority as compared to 31 other patients followed up for a similar length of time and who required dapsone. IgA deposits were still present in the skin of these patients, although in a state of clinical remission. There was no suggestion of a decrease in the quantity of IgA in these 3 patients, and C₃ was present in one of them whilst in clinical remission.

Why IgA deposits are found in the skin in patients with DH is not known. In itself it cannot be related to gluten sensitive enteropathy as it is not present in patients with coeliac disease (Seah *et al.*, 1972). The factors which predispose to gluten sensitive enteropathy in DH and coeliac disease are in all probability similar, but in DH there appears to be an additional abnormality, either of IgA or reticulin, which allows for the deposition of IgA in the skin. However, this deposition *per se* does not appear to result in skin lesions as the IgA persists in patients controlled by a GFD and in those who are in spontaneous clinical remission.

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REFERENCES

- FRY, L. & SEAH, P.P. (1974) Dermatitis herpetiformis: an evaluation of diagnostic criteria. *British Journal of Dermatology*, **90**, 137.
- HARRINGTON, C.I. & READ, N.W. (1977) Dermatitis herpetiformis: effect of gluten-free diet on skin IgA and jejunal structure and function. *British Medical Journal*, **i**, 872.
- JABLONSKA, S., CHORZELSKI, T.P., BEUTNER, E.H. & HOLUBAR, K. (1973) Indications for skin and serum immunofluorescent studies in dermatology. In: *Immunopathology of the Skin: Labelled Antibody Studies* (Ed. E.H. Beutner, T.P. Chorzelski, S.F. Bean and R.E. Jordan), p. 13. Dowden, Hutchinson & Ross Inc., Stroudsburg, Pennsylvania.
- MOHAMMED, I., HOLBOROW, E.J., FRY, L., THOMPSON, B.R., HOFFBRAND, A.V. & STEWART, J.S. (1976) Multiple immune complexes and hypocomplementaemia in dermatitis herpetiformis and coeliac disease. *Lancet*, **ii**, 487.
- SAMS, W.M., JR (1974) Pemphigus and pemphigoid. In: *Immunological Aspects of Skin Diseases* (Ed. by L. Fry and P.P. Seah), p. 7. Medical and Technical Publishing Co. Lancaster.
- SEAH, P.P. & FRY, L. (1975) Immunoglobulins in the skin in dermatitis herpetiformis and their relevance in diagnosis. *British Journal of Dermatology*, **92**, 157.
- SEAH, P.P., FRY, L., STEWART, J.S., CHAPMAN, B.L., HOFFBRAND, A.V. & HOLBOROW, E.J. (1972) Immunoglobulins in the skin in dermatitis herpetiformis and coeliac disease. *Lancet*, **i**, 611.

In vitro effects of pemphigus antibodies on skin

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SUMMARY

Acantholysis occurring in rhesus monkey skin explants cultured on sera of 9 pemphigus patients was found to be largely dependent on the titre of intercellular antibody, and not on the participation of complement. Skin explants cultured on normal human sera and pemphigoid sera failed to give rise to intercellular staining or to develop lesions.

Six of eight 'negative' pemphigus sera with intercellular antibody titres of less than 20 on skin (and titres ranging from 20 to 160 on monkey oesophagus) reacted with the skin explants as revealed by direct immunofluorescence with an anti-IgG conjugate. The binding of antibodies from 3 of these 6 reactive sera resulted in some pathological changes in the explants. At least two of these 3 'negative' sera came from pemphigus patients with skin lesions.

Pemphigus antibodies bind to their antigen *in vivo* as suggested by the fixation of IgG to the intercellular (IC) substance of the epidermis and oral mucosa in and around bullae of patients (Beutner & Jordon, 1964; Beutner *et al.*, 1965) and in tissue culture produce 'lesions' in skin explants maintained on pemphigus sera (Michel & Ko, 1974; Schiltz & Michel, 1976; Deng *et al.*, 1977; Binder *et al.*, 1976; Barnett *et al.*, 1977). This binding reaction and the formation of intra-epidermal acantholytic 'lesions' in skin explants afford a means of investigating a common discrepancy between the development of epidermal bullae and the failure of the patients' sera to yield indirect immunofluorescent staining reactions with epidermis.

MATERIALS AND METHODS

The culture system employed differed from those used previously in that the rhesus monkey skin explants were cultured on sterilized discs of Whatman filter paper supported by a stainless steel grid instead of on rafts of lens papers. Thus, explants could be handled by grasping the edge of the filter paper disc and artifactual trauma through mechanical manipulation was minimized.

Explants were cultured on whole human sera at 31°C in a moist chamber with a 5% CO₂ and air mixture. Pemphigus sera which contained IC antibodies reactive either with IC antigens of both

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monkey skin and oesophageal mucosa, or with only the latter, as determined by indirect immunofluorescence, served as culture media. Explants were also cultured on bullous pemphigoid sera and normal human sera as a control.

The characteristics of the conjugates employed in this study were as follows: C-458, anti-human IgG, molar F/P = 2.1, antibody = 4.52 mg/ml, 16 units, use dilution = 1/180; C-461, anti-C4, F/P = 2.46, 8 units, use dilution = 1/32; C-413, anti-C3, F/P = 2.36, antibody = 0.66 mg/ml, use dilution = 1/32; C-338, anti-IgM, F/P = 3.65, 4 units, working dilution = 1/16.

The optical system for the immunofluorescent study consisted of a Model 10 AO Fluorolume microscope with a mercury vapour lamp (HBO 200W) with an AO 5840 exciter filter. A darkfield condenser with toric lens was used.

RESULTS

Antibody binding and kinetics

The kinetics of antibody binding in this system were the same as reported by Deng *et al.* (1977).

Complement immunofluorescence

C3 and C4 complement components were not detected in any of the explants cultured in pemphigus sera by direct immunofluorescence although the same techniques had revealed large quantities of IgG bound to the IC areas of the skin. These same IgG antibodies were further shown to be incapable of binding complement by an indirect complement immunofluorescence test. The IgG antibodies bound to the BMZ of explants cultured in the two reactive pemphigoid patient sera, however, did bind complement components C3 and C4 in culture.

Cytopathic effect

The acantholytic effect varied with the titre of the pemphigus antibodies in sera that served as culture media (Fig. 1). All 9 sera with IC antibody titres of 80 or greater on skin produced such acantholytic changes. These differences also manifested themselves by '*in vitro* Nikolsky signs' whereby the roof of the intra-epithelial lesions could be lifted off with forceps. Histologically, both the roof and the floor contained adherent epidermal cells. Only the stratum corneum could be pulled off from some of the skin explants cultured on control sera. Explants cultured in normal human sera retained a normal IC apposition. Explants cultured in each of the five pemphigoid sera also retained their normal appearance though antibodies from two of them bound to the BMZ of the explants.

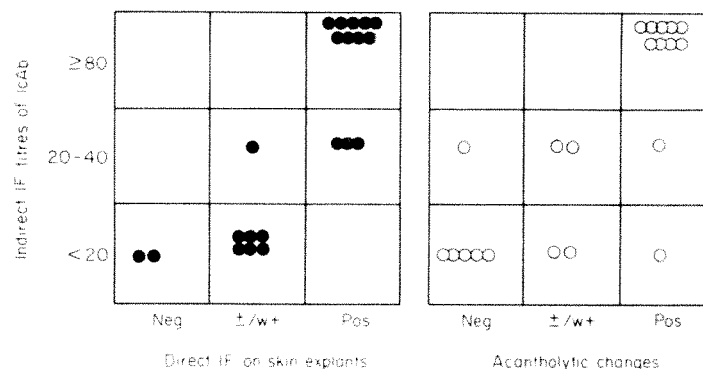


FIGURE 1. Correlation of intercellular antibody titre with direct immunofluorescence and acantholytic changes in cultured skin explants.

All four of the sera with IC antibody titres of 20–40 gave direct immunofluorescence reactions in the organ culture test system; only three of them gave rise to detectable acantholytic changes. Of these, one produced marked acantholysis and two induced only minimal specific change. Eight pemphigus sera gave negative indirect immunofluorescence reactions on monkey skin (titres of less than 20) while IC antibody titres on monkey oesophagus and/or gingiva ranged from 20 to 160. Interestingly, six of these eight sera, nevertheless, did react with monkey skin in organ culture as revealed by $\pm/w+$ direct IF reactions; and three of these induced some minimal acantholytic changes.

Three of these pemphigus sera which gave negative indirect immunofluorescent reactions on skin sections came from patients who had skin lesions. Two of these three sera gave rise to positive direct immunofluorescence of the intercellular area.

DISCUSSION

Pemphigus antibodies in whole sera, or IgG fractions thereof, are capable of binding to skin explants and of producing acantholytic changes in them similar to those of the human lesion (Michel & Ko, 1974; Schiltz & Michel, 1976; Deng *et al.*, 1977 and this report). This acantholytic effect is dependent on the titre of the ICAb, a finding which implicates these antibodies in the pathogenesis and compares with the titres of serum antibodies paralleling disease activity (Beutner *et al.*, 1970).

Our data and those of others (Schiltz *et al.*, 1976) indicate that these IgG antibodies cause cytopathic changes without the involvement of complement, as has been found in other studies (Loewi, 1963; Ehrlich *et al.*, 1962; Oda & Puck, 1960; Dumond *et al.*, 1961).

Interestingly, some of the 'negative' sera (titres of less than 20 on skin but positive on monkey oesophagus) reacted weakly with cultured skin explants, three of them producing acantholytic changes. Cormane & Petzoldt (1970) reported several cases in which immunofluorescence tests of patient sera on human skin were negative, yet tests performed with other antigenic substrates were found to be positive for pemphigus antibodies as in our own findings on rabbit oesophagus (Chorzelski & Beutner, 1969).

One possible explanation for discrepant observations on some of our sera is that the antibodies are present and can induce lesion formation but cannot be detected by the standard indirect immunofluorescence tests if they are done on skin sections. Another explanation is that the high affinity serum antibodies are at least in part absorbed by reacting with skin *in vivo*. Whatever the explanation for the observed discrepancies we disagree with those who suggest that pemphigus antibodies have no pathological significance (Parish, 1971).

REFERENCES

- BARNETT, M.L., BEUTNER, E.H. & CHORZELSKI, T.P. (1977) Organ culture studies of pemphigus antibodies. II. Ultrastructural comparison between acantholytic changes *in vitro* and human pemphigus lesions. *Journal of Investigative Dermatology*, **68**, 265.
- BEUTNER, E.H., CHORZELSKI, T.P. & JORDON, R.E. (1970) Clinical significance of pemphigus antibodies. In: *Autosensitization in Pemphigus and Bullous Pemphigoid* (Ed. by E.H. Beutner, T.P. Chorzelski & R.E. Jordon), p. 69. Charles C. Thomas, Springfield, Illinois.
- BEUTNER, E.H. & JORDON, R.E. (1964) Demonstration of skin antibodies in sera of pemphigus vulgaris patients by indirect immunofluorescent staining. *Proceedings of the Society for Experimental Biology and Medicine*, **117**, 505.
- BEUTNER, E.H., LEVER, W.F., WITEBSKY, E., JORDON, R.E. & CHERTOCK, B. (1965) Autoantibodies in pemphigus vulgaris: Response to an intercellular substance of epidermis. *Journal of the American Medical Association*, **192**, 682.
- BINDER, W.L., BEUTNER, E.H., SHU, S. & BARNETT, M.L. (1976) *In vitro* effects of pemphigus antibodies on skin and gingiva. *Journal of Dental Research*, **55**, B299.

- CHORZELSKI, T.P. & BEUTNER, E.H. (1969) Factors contributing to occasional failures in demonstration of pemphigus antibodies by the immunofluorescence test. *Journal of Investigative Dermatology*, **53**, 188.
- CORMANE, R.H. & PETZOLDT, D. (1970) Immunofluorescence studies on the affinity of pemphigus antibodies to epithelial intercellular substances. *Dermatologica*, **140**, 1.
- DENG, J.S., BEUTNER, E.H. & SHU, S. (1977) Organ culture studies of pemphigus antibodies. I. Studies of antigen stability in explants of normal monkey skin and of kinetics of acantholytic changes. *Archives of Dermatology*, **113**, 923.
- DUMOND, D.C., WALTER, C.M., BITENSKY, L., CUNNINGHAM, G.J. & CHAYEN, J. (1961) Intracellular response to an iso-immune reaction at the surface of ascites tumor cells. *Nature*, **192**, 1302.
- EHRlich, G., HALBERT, S.P. & MANSKI, W. (1962) Cytotoxicity of anti-tissue antibodies. II. Effects of anti-lens sera in tissue culture. *Journal of Immunology*, **89**, 391.
- LOEWI, G. (1963) Effect of antibody on heterologous and homologous cells in tissue culture. *Immunology*, **6**, 569.
- MICHEL, B. & KO, C.S. (1974) Effect of pemphigus or bullous pemphigoid sera and leukocytes on normal human skin in organ cultures. An *in vitro* model for the study of bullous diseases. *Journal of Investigative Dermatology*, **62**, 541.
- ODA, M. & PUCK, T.T. (1960) The interaction of mammalian cells with antibodies. I. *Journal of Experimental Medicine*, **113**, 599.
- PARISH, W.E. (1971) General concepts of auto-sensitivity in disease. In: *Immunology and the Skin* (Ed. by W. Montagna and R.E. Billingham), p. 233. Appleton-Century-Crofts, New York.
- SCHILTZ, J.R. & MICHEL, B. (1976) Production of epidermal acantholysis in normal human skin *in vitro* by the IgG fraction from pemphigus serum. *Journal of Investigative Dermatology*, **67**, 254.

Cellular immunity to *P. acnes* in the normal population and patients with acne vulgaris

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SUMMARY

Patients with varying degrees of acne, acne-free adult controls and samples of cord blood were investigated for cell mediated immunity to *P. acnes* using a leukocyte migration inhibition test. Despite the fact that the mean migration index tended to decrease with acne severity, only the patients with severe acne showed cell-mediated immunity. It is suggested that when cellular immunity arises it is a late event which may contribute to inflammation but is probably not a factor in its initiation.

In all probability, acne vulgaris both aetiologically and pathologically is a multifactorial disease. Decades of research have as yet failed to elucidate the exact mechanisms which trigger the development of the characteristic initial lesions and those factors which decide the type and magnitude of the subsequent inflammatory response. Since *Propionibacterium acnes* (Group 1 of Marples & McGinley, 1974) is now generally agreed to be intimately associated with acne vulgaris and can be readily isolated from both acne lesions and normal skin (Puhvel, Reisner & Amirian, 1975; Marples & Izumi, 1970), cell mediated immunity (CMI) to this organism might play an important role in (a) the development of and/or (b) the persistence of the inflammatory response in acne vulgaris.

Few papers of note have been published which have any bearing on CMI to *P. acnes* and acne vulgaris. Puhvel *et al.* (1967) failed to demonstrate delayed hypersensitivity skin reactions to the organism in either normals or acne patients; and Puhvel *et al.* (1977) demonstrated that peripheral blood mononuclear cells (predominantly lymphocytes) from patients with nodulo-cystic acne could be stimulated to transform by 'bacterial extracellular products' prepared from culture supernatants of *P. acnes* whereas cells from acne-free adults could not.

The present experiments were undertaken in an attempt to demonstrate CMI to *P. acnes* in both the normal population and patients with acne of varying severity using formalin-killed, washed *P. acnes* as the antigen and the leukocyte migration inhibition test as an indicator of CMI.

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MATERIALS AND METHODS

Normal population

This comprised 28 individuals (14 of each sex) whose ages ranged from 18 to 30 years. Males and females were matched as nearly as possible for age. All were acne-free at the time of investigation and professed no past history of the condition.

Acne patients

All the patients investigated (apart from some of those classified as having 'mild' acne) were drawn from attenders at the dermatology clinic in Leeds; none was on any medication at the time of investigation nor had taken any over the previous 6 weeks. Patients were graded according to the method of Burton *et al.* (1971). Those with a score of 2 were classified as having mild acne; 3 moderate; and 4 or 5 severe. All the patient groups had the same age range as the controls, contained equal numbers of males and females and were age matched as nearly as possible. The numbers in these groups are shown in Table 1.

TABLE 1. Migration inhibition indices of peripheral blood leukocytes from cord blood, acne-free adults and patients with acne vulgaris of varying severity

Leukocyte source	Acne grade	No. tested	Age $\pm$ s.d. (years)	Mean migration index $\pm$ s.d.
Cord blood	—	15	—	0.98 $\pm$ 0.06
Normal adults	Nil	28	22.0 $\pm$ 3.3	0.93 $\pm$ 0.06
Acne patients	Mild	26	21.5 $\pm$ 3.5	0.89 $\pm$ 0.06
Acne patients	Moderate	22	22.8 $\pm$ 4.0	0.86 $\pm$ 0.07
Acne patients	Severe	22	22.6 $\pm$ 3.8	0.78 $\pm$ 0.13

In addition, information was obtained from 2 smaller groups (a) a group of eight 13–14-year-old children with severe acne and (b) a group of eight acne-free members of the Department of Dermatology (age range 18–35). This latter group, made up of nursing and research staff, although acne-free, had come into frequent contact with acne patients or had been isolating *P. acnes* from patients and working with the organism for some years.

Preliminary experiments and other workers (e.g. Johnson & Cummins, 1972) have shown that *P. acnes* forms a large homology group, the members of which are intimately related antigenically. An antiserum to a typical member of this group will therefore agglutinate the vast majority of other members to titre. The organism used as the antigen in the migration inhibition test was a typical *P. acnes* (Group 1 of Marples & McGinley, 1974) isolated from a blackhead lesion on a patient in Leeds. This organism (Laboratory no. P-37) was selected because a great deal of background information existed (Roberts, 1975). It was grown in 2 litres of Reinforced Clostridial Medium (Oxoid) for 48 h at 37°C. Sterile nitrogen was continually bubbled through the medium to provide anaerobiosis and constant agitation. After 48 h the culture was centrifuged at 3000 g for 30 min, then washed $\times 4$ in PBS pH 7.5 (Dulbecco A, Oxoid). Finally a thick suspension (2×10^{11} organisms per ml) was made in PBS and 1% by volume 40% formaldehyde added. For use in the test, this suspension was washed twice in PBS to remove excess formalin.

Migration inhibition test

The test used was basically that of Federlin *et al.* (1971) using TC199 (100,000 units of penicillin and 200 mg of streptomycin sulphate per ml) with 1% human serum albumin added. Albumin was chosen to eliminate any possible effects of antibodies to *P. acnes* which are present in human serum.

Using leukocytes obtained from 5 samples of cord blood, experiments were performed to ascertain that concentration of antigen which was non-toxic. A final concentration of 10^8 *P. acnes* per ml was the highest non-toxic concentration and was therefore used in all further tests. Results were expressed as:

$$\text{Migration Index (MI)} = \frac{\text{area of migration with antigen present}}{\text{area of migration without antigen}}$$

Non-sensitized population (cord blood)

In an attempt to obtain an absolute baseline the leukocytes from 15 samples of cord blood were tested as being representative of a population which in theory should have no CMI to *P. acnes*.

RESULTS

The results obtained from cord blood, normal adults and acne patients are presented in Fig. 1 and Table 1. If the cord blood results are taken as an absolute baseline then a migration index of below 0.86 (mean $\pm$ 2 s.d.) would indicate sensitization. Thus, as a group, only the patients with severe acne could be classified as sensitized, i.e. showing CMI to *P. acnes*. As individuals, 2/28 normal adults, 7/26 mild, 9/22 moderate and 16/22 severe acne patients would show some degree of CMI.

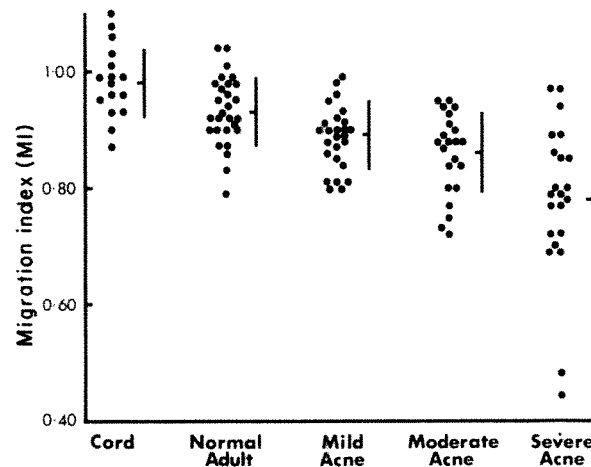


FIGURE 1. Diagram showing the relationship between migration index and acne grade when peripheral blood leukocytes are tested against *P. acnes* in a leukocyte migration inhibition test. The bar represents the mean and the vertical line $\pm$ one standard deviation.

However, with this particular test, it is not customary to take results obtained with cord blood as the baseline against which to judge the degree of sensitization. With almost all the bacterial antigens used it is usual to take a migration index of <0.80 as indicating sensitivity. The result obtained here with the acne-free adults (0.93 ± 0.06) would give a lower limit of 0.81, i.e. for practical purposes identical with the figure in universal use. Using the latter as the criterion for sensitization only the patients with severe acne taken as a group show CMI to *P. Acnes*. Individually 4/22 moderates and

12/22 severes were positive. However, very few of these (e.g. 2/22 severes) could be classified as highly sensitized.

The group of 13-14-year-olds with severe acne gave a result which approximated to that obtained with cord blood (see Table 2) whilst the adult, acne-free members of the Department of Dermatology as a group bordered on sensitization (0.81 ± 0.09).

TABLE 2. Migration indices in children with severe acne and acne-free dermatologists

Leukocyte source	Acne grade	No. tested	Mean migration index $\pm$ s.d.
13-14-year-olds	Severe	8	0.97 ± 0.09
Adults, Dermatology Dept.	Nil	8	0.81 ± 0.08

DISCUSSION

Our finding that, as a group, only the patients with severe acne showed significant CMI to *P. acnes* appears to be in general agreement with the results of Puhvel *et al.* (1977). These authors showed that the lymphocytes of a group of patients with nodulo-cystic acne would transform in the presence of 'lyophilized bacterial extracellular products' from a culture of *P. acnes* to a significantly greater extent than those from adult control subjects. However, further comparison is not possible as results for individuals were not quoted.

It is clear from Fig. 1 that the fact that a person has severe acne is no guarantee that they will be sensitized to *P. acnes*; the group showing considerable variability ranging from no CMI at all to a high degree of sensitivity. This might indicate that not only is the degree of CMI variable but also that its development might be a somewhat unpredictable event.

That the development of CMI to *P. acnes* does not necessarily depend on the severity of the condition *per se* is evidenced by the results from the group of 13-14-year-olds with severe acne. As a group they gave a result almost identical to that observed using cord blood, i.e. they showed no sensitivity at all. There is evidence however that CMI may be related to the length and level of exposure to the organism. Two pieces of evidence might point to this. First, the steady decline in the mean migration index with the severity of acne and second, that a group of acne-free workers in the Dermatology Department, servicing acne clinics gave a mean migration index bordering on significance (Table 2). All the foregoing of course would be influenced by whether the individual was a good or a poor responder to the types of immunogen associated with the organism. It should also be remembered that susceptibility to infection with an organism does not always mean that CMI will develop during or following an infection.

The fact that we were able to demonstrate CMI in people with severe acne but only a high degree of sensitivity in very few might have some bearing on the results of Puhvel *et al.* (1967). These authors failed to demonstrate delayed hypersensitivity skin reactions to the organism in both controls and severe acne cases. If a reasonable degree of CMI (detectable in the peripheral circulation) is necessary before a patient becomes skin test sensitive, then our results might indicate that delayed hypersensitivity skin reactions might be uncommon.

In general we would agree with the statement of Puhvel *et al.* (1977) that 'the involvement of the host's immunological response to *P. acnes* should be reconsidered as a possible contributing factor in

the inflammatory response in acne'. We would consider that CMI to *P. acnes* might play a role in the inflammatory response around lesions (especially those progressing to chronicity) but that it would occur late in the chain of events and it would be unlikely to be one of the initiating factors.

ACKNOWLEDGMENTS

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REFERENCES

- BURTON, J.L., CUNLIFFE, W.J., STAFFORD, I. & SHUSTER, S. (1971) The prevalence of acne vulgaris in adolescence. *British Journal of Dermatology*, **85**, 119.
- FEDERLIN, K., MAINI, R.N., RUSSELL, A.S. & DUMONDE, D.C. (1971) A micro-method for peripheral leucocyte migration in tuberculin sensitivity. *Journal of Clinical Pathology*, **24**, 533.
- JOHNSON, J.L. & CUMMINS, C.S. (1972) Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical *Propionibacteria* and strains of *Arachnia propioriea*. *Journal of Bacteriology*, **109**, 1047.
- MARPLES, R.R. & IZUMI, A.K. (1970) Bacteriology of pustular acne. *Journal of Investigative Dermatology*, **54**, 252.
- MARPLES, R.R. & MCGINLEY, K.J. (1974) *Corynebacterium acnes* and other anaerobic diphtheroids from human skin. *Journal of Medical Microbiology*, **7**, 349.
- PUHVEL, S.M., HOFFMAN, K., RESINER, R.M. & STERNBERG, T.H. (1967) Dermal hypersensitivity of patients with acne vulgaris to *Corynebacterium acnes*. *Journal of Investigative Dermatology*, **49**, 154.
- PUHVEL, S.M., RESINER, R.M. & AMIRIAN, D.A. (1975) Quantitation of bacteria in isolated pilosebaceous follicles in normal skin. *Journal of Investigative Dermatology*, **65**, 525.
- PUHVEL, S.M., AMIRIAN, D., WEINTAUB, J. & REISNER, R.M. (1977) Lymphocyte transformation in subjects with nodulo-cystic acne. *British Journal of Dermatology*, **97**, 205.
- ROBERTS, C.D. (1975) The role of bacteria in acne vulgaris. Ph.D. Thesis, University of Leeds, Department of Microbiology.

Acid hydrolases in blister fluid

3. CHARACTERIZATION AND QUANTIFICATION OF PEPTIDE HYDROLASES

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SUMMARY

Cathepsin B₁ and Cathepsin C have been characterized and quantified in suction blister fluid. These enzymes revealed practically no activity in interstitial fluid and serum.

Arylamidase was also investigated and found to have similar concentrations in serum, blister fluid and interstitial fluid. The properties of arylamidase from these sources were identical.

Hydrolysis of proteins to peptides and amino acids is an important function of lysosomes. Coffey & de Duve (1968) have documented the capacity of lysosomes to degrade proteins to dipeptides and amino acids. A great deal of information is now available regarding the properties of the individual enzymes catalyzing this process.

Cathepsin B₁ possesses both exo- and endopeptidase activity. The enzyme degrades collagen (Burleigh, Barret & Lazarus, 1974), proteoglycan (Morrison *et al.*, 1973) and benzoylarginine-2-naphthylamide at pH 6 (Jansén & Hopsu-Havu, 1969). Cathepsin C is an exopeptidase that hydrolyzes peptides, especially at bonds involving an aromatic amino acid adjacent to a free α -amino group. Since the enzyme can liberate dipeptides and since a free α -amino or α -imino group on the substrates is required, the enzyme may be classified as an aminopeptidase (and iminopeptidase), and the name dipeptidyl aminopeptidase I has been used. The normal physiological substrate of the arylamidases is unknown, though they are commonly regarded as peptide hydrolases. The arylamidases cleave amino acids naphthylamides. Both cathepsin B₁ and cathepsin C were found to require thiol groups for full activity (Mier & van den Hurk, 1975), the reaction rates being less than 20% of maximal in the absence of cysteine or cysteamine.

Analogous to preceding papers (Volden, 1978), this paper describes kinetic properties and possible factors influencing the reaction of some peptide hydrolases in suction blister fluid. Where possible, results are compared with those of interstitial fluid and serum.

MATERIALS AND METHODS

The fluorogenic substrates α -N-benzoyl-arginine- β -naphthylamide HCl, glycyl-L-phenylalanine- β -naphthylamide, α -leucyl- β -naphthylamide HCl and β -naphthylamine (NA) were purchased from

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the Sigma Chemical Company. The method of raising suction blisters and the collection of interstitial fluid are described in a previous paper (Volden, 1978). The assay procedures are principally the same as used by Mier & van den Hurk (1975) for guinea-pig epidermis.

Measurements of enzymic activity

The enzymes were assayed at 37°C at their optimal pH by the rate of liberation of β -NA from the appropriate substrates. The routine assay mixture contained 0.1 ml suction blister fluid, interstitial fluid or serum and 0.1 ml of the buffered substrate. Concentrations of buffered substrates were for cathepsin B₁ 2.0 mmol/l, for cathepsin C and arylamidase 0.4 mmol/l. Phosphate (100 mmol/l) EDTA (1.2 mmol/l) and cysteamine (4.0 mmol/l) buffer was used for cathepsin B₁ and cathepsin C while phosphate (100 mmol/l) buffer was used for arylamidase. Reactions were incubated 30 min before stopping by the addition of 1.0 ml of carbonate/bicarbonate buffer (pH 10.5). The fluorescence ($\lambda_{\text{ex}}=312$ nm, $\lambda_{\text{em}}=415$ nm) was read within a few minutes.

Linear calibration curves were obtained for β -NA concentrations 0.01–1.0 nmol/l in the appropriate buffer. The standard consisted of β -NA 0.1 mmol/l in carbonate/bicarbonate buffer (pH 10.5).

Effect of reaction variables

The following reaction variables were investigated: time of incubation from zero to 75 min, effect of pH, effect of substrate concentration, sensitivities to 5 mmol/l concentrations of Na tartrate, MgCl₂, CaCl₂, KCl and NaF, effects of DMSO, and heat inactivation at 56°C for 5 and 30 min. Blister fluid was diluted with water in the range 1:1 up to 1:10 in order to confirm that activity was proportional to enzyme concentration over the range employed. The enzyme activity was studied with respect to changes in protein concentration and cellular content of the blister fluid and interstitial fluid (Volden, 1978).

RESULTS

The excitation curve showed two peaks, the higher at 312 nm and the smaller at 335–340 nm. A reading of at least double the blank (fluorescence of substrate and of blister fluid, interstitial fluid or serum) was required for accurate quantification of enzyme activity. The reaction reading was less than double the blank for cathepsin B₁ and cathepsin C in the cases of interstitial fluid and serum, and just double the blank in the case of blister fluid. The blank was about 20% of the activity of arylamidase.

The reaction rates were linear with dilution of blister fluid down to 1:2 for cathepsin B₁ and C, and down to 1:10 for arylamidase.

No correlation of any of the investigated enzymes with time taken for bulla formation was found.

As described earlier (Volden, 1978), the total protein concentration of blister fluid varied between 14 and 26 g/l. The total number of erythrocytes and leukocytes per mm³ were respectively 0–10 and 0–20. The enzyme activity of each of the three enzymes did not show any significant correlation with protein concentration or number of cells in suction blister fluid.

Effect of incubation time

Enzyme activity was proportional to incubation time for cathepsin B₁ and cathepsin C of blister fluid for about 30 min, whereafter the activity declined. The progress curves of arylamidase from blister fluid, interstitial fluid and serum were linear for at least 90 min.

Effect of pH

The pH optima of the different enzymes are listed in Table 1. The same pH optimum was found for arylamidase in blister fluid, interstitial fluid and serum.

TABLE 1. pH optima of peptidases of blister fluid, interstitial fluid and serum

Enzyme	Investigated pH range	pH optimum
Cathepsin B ₁	4.2-7.7	6.4 (only blister fluid)
Cathepsin C	3.3-6.5	5.2 (only blister fluid)
Arylamidase	6.4-9.0	7.6

TABLE 2. Michaelis-Menten constants of the peptidases

Enzyme	K _m for substrate		
	Blister fluid	Interstitial fluid	Serum
Cathepsin B ₁	7.0×10^{-3} mol/l		
Cathepsin C	2.5×10^{-4} mol/l		
Arylamidase	3.0×10^{-4} mol/l	3.3×10^{-4} mol/l	3.2×10^{-4} mol/l

Effect of substrate concentration

Lineweaver and Burk plots of cathepsin B₁ and cathepsin C in blister fluid and of arylamidase in blister fluid, interstitial fluid and serum were linear over the range of substrate concentration investigated (0.5-4.0 mmol/l for cathepsin B₁ and 0.04-0.8 mmol/l for cathepsin C and arylamidase). The Michaelis-Menten constants (K_m values) are listed in Table 2.

Effect of heat inactivation

Cathepsin B₁ lost 36% of its activity after heating at 56°C for 30 min. Cathepsin C was rather thermostable while arylamidase lost about 30% of its activity after the same heating procedure.

Sensitivity to activators and inhibitors

None of the investigated substances had any significant influence on the enzyme activity. DMSO inhibited arylamidase slightly (8%) at high concentration (8% DMSO). Cathepsin B₁ and cathepsin C were not inhibited by DMSO at concentrations up to 8%.

Normal values

The enzymatic activity is expressed as the rate of β-NA liberated (pmol/min/ml). Relatively similar

TABLE 3. Activities of peptidases. The levels of cathepsins B₁ and C in interstitial fluid and serum were too low to permit reliable quantification. The mean activity of 6 assays is expressed as pmol/min/ml. Range in parentheses

Enzyme	Blister fluid	Interstitial fluid	Serum
Cathepsin B ₁	25 (11-65)		
Cathepsin C	15 (4-38)		
Arylamidase	130 (94-160)	110 (75-150)	140 (94-168)

values were found for the activities of arylamidase in blister fluid, interstitial fluid and serum (Table 3). Cathepsin B₁ and cathepsin C revealed very low activity in blister fluid and practically no activity in interstitial fluid and serum.

DISCUSSION

Most authors measure β -NA at an excitation wavelength round 335 nm (Mier & van den Hurk, 1975; Lumen & Tappel, 1972). In this study two peaks were found and, unlike the results of other investigators, the highest peak was found at 312 nm. A possible explanation may be that the fluorometer used in the present study is relatively more sensitive at the shorter wavelengths compared with those used by other workers.

This study clearly shows for arylamidase that the same enzyme is present in blister fluid, interstitial fluid and serum. Very low activity was found for cathepsin B₁ and cathepsin C. Starkey & Barrett (1973) found that cathepsin B₁ was inhibited by normal human serum, the major inhibitor present in serum being α_2 -macroglobulin.

Good agreement with other investigators (Mier & van den Hurk, 1975; Hopsu-Havu & Jansén, 1969) was found for the enzyme characteristics of cathepsin B₁ and cathepsin C. The Michaelis-Menten constants of arylamidase correspond well with the value (3.5×10^{-4} mol/l) found by de Bersaques (1968) for human epidermis, whereas Mier & van den Hurk (1975) reported lower values for guinea-pig epidermis. The results of thermal inactivation of cathepsin B₁ are quite similar to those of Ali *et al.* (1967), who found a loss of about 50% of its activity after heating at 52°C for 30 min.

According to previous papers (Volden, 1978) the percentage to each enzyme which leaks into the blister fluid was calculated. There was practically no activity of cathepsin B₁ and cathepsin C in interstitial fluid. The calculation therefore roughly gives the leakage of each of these enzymes into the blister fluid. This was less than 4% of the activity in intact epidermis.

In summary, it appears that all three of the enzymes reported in the present paper could be of value as an index of lysosomal damage. The use of the suction blister as a model for investigating the effects of UVR on lysosomes *in vivo* will be described in the following report.

REFERENCES

- ALI, S.Y., EVANS, L., STAINTHORPE, E. & LACK, C.H. (1967) Characterization of cathepsins in cartilage. *Biochemical Journal*, **105**, 549.
- BERSAQUES, J.DE (1968) Leucine aminopeptidase et naphthylamidases dans l'épiderme humain. LI. Donees biochimiques. *Enzymologia Biologica et Clinica*, **9**, 219.
- BURLEIGH, M.C., BARRETT, A.J. & LAZARUS, G.S. (1974) Cathepsin B₁, a lysosomal enzyme that degrades native collagen. *Biochemical Journal*, **137**, 387.
- COFFEY, J.W. & DE DUVE, C. (1968) Digestive activity of lysosomes. I. The digestion of proteins by extracts of rat liver lysosomes. *Journal of Biological Chemistry*, **243**, 3255.
- HOPSU-HAVU, V.K. & JANSÉN, C.T. (1969) Peptidases in the skin. II. Demonstration and partial separation of several specific dipeptide naphthylamidases in the rat and human skin. *Archiv für Klinische und Experimentelle Dermatologie*, **235**, 53.
- JANSÉN, C.T. & HOPSU-HAVU, V.K. (1969) Proteolytic enzymes in the skin. II. A comparative study of skin homogenates of five mammalian species. *Acta dermato-venereologica*, **49**, 468.
- LUMEN, B.O. DE & TAPPEL, A.L. (1972) Continuous fluorometric assay of cathepsin B-like activity in lysosomes. *Analytical Biochemistry*, **48**, 378.
- MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975) Lysosomal hydrolases of the epidermis. III. Peptide hydrolases. *British Journal of Dermatology*, **93**, 509.
- MORRISON, R.I.G., BARRETT, A.J., DINGLE, J.T. & PRICE, D.C. (1973) Cathepsin B₁ and D. Action on human cartilage proteoglycans. *Biochimica et Biophysica Acta*, **302**, 411.
- STARKEY, P.M. & BARRETT, A.J. (1973) Human cathepsin B₁. Inhibition by α_2 -macroglobulin and other serum proteins. *Biochemical Journal*, **131**, 823.
- VOLDEN, G. (1978) Acid hydrolases in blister fluid. 1. Characterization and quantification of acid phosphatase. 2. Characterization and quantification of glycoside hydrolases. *British Journal of Dermatology*, **98**, 273 & 281.

Acid hydrolases in blister fluid

4. INFLUENCE OF ULTRAVIOLET RADIATION

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SUMMARY

Large, dose-related increases have been demonstrated in the activity of lysosomal hydrolases in the fluid of experimental suction blisters raised on skin previously irradiated with UVR-B. These increases appear after a latent period of 11–18 h, and the pattern of changes shows an extremely good correlation with the hydrolase profile of epidermis.

These observations offer strong support for the concept that lysosomes participate in the sequence of events following UV irradiation, but seem to rule out the lysosome as the initial target for UVR.

A great deal has been published regarding the reaction of skin to ultraviolet radiation (UVR). Surprisingly, however, the precise chain of events leading to the visible signs of sunburn (erythema, oedema, and so on) is still obscure. The possibility that lysosomes may play a role in this process was first suggested by the experiments of Weissman & Dingle (1961), and of Weissman & Fell (1962) and has since been discussed in detail by other authors (Johnson & Daniels, 1969; Lazarus & Hatcher, 1975). Clearly, the demonstration that UVR can induce lysis of lysosomal preparations *in vitro* (Desai, Sawant & Tappel, 1964) is far from proof that such an event occurs *in vivo*; a further possibility that lysosomal rupture is actually the initial event in the sequence is merely speculative.

We have therefore employed the suction blister as a model to obtain more data about the response of normal skin to UVR; the advantages and limitations of this approach have already been discussed (Volden, 1978a). In this report we describe the effects of prior irradiation on the levels of 8 lysosomal hydrolases in blister fluid.

MATERIALS AND METHODS

General test procedure

All experiments were carried out on the abdominal skin of healthy volunteers aged 17–76. Test sites (about 1 cm²) were irradiated with UVR-B, UVR-A or visible light using an Osram High Pressure Xenon Arc Lamp (XBO=150W) equipped with the filter combination shown in Table 1.

At an appropriate time after irradiation, a suction cup was placed over the test site, care being taken to locate the bore in the adaptor plate exactly over the exposed skin. A suction bulla was raised as described previously (Volden, 1978a), except for the minor differences in suction times and

TABLE 1. Details of filters, wavelengths and radiation intensities employed. Filter 310 is interference filter, the others are cut-off filters. Transmission characteristics are measured spectrophotometrically (Volden & Thune, 1977), energy output is measured prior to each exposure by a Hewlett and Packard Radiant Flux Meter

Region	Schott filters	Range of wavelength		Intensity (mW.cm ⁻²)	Total doses (J.cm ⁻²)
		Half bandwidth	Tenth bandwidth		
UVR-B	KG ₁ +310	305-315 nm	301-317 nm	1.2	0.08-2.0
UVR-A	KG ₁ +WG ³²⁰ + UG ⁵	350-390 nm		9	25
Visible	KG ₁ +GG ⁴⁹⁵	495-745 nm		80	60

pressures indicated in the results section below. Blister fluid was collected as described before, and enzyme assays were either performed immediately or the sample stored at -20°C prior to analysis.

The following enzymes were determined in all samples; acid phosphatase, α -glucosidase, α - and β -galactosidase, α -mannosidase, β -acetylglucosaminidase, β -glucuronidase and arylamidase. Enzyme assays were exactly as described previously (Volden, 1978a-c). The determination of protein and cellular content of blister fluid after irradiation was made according to a previous report (Volden, 1978a). For this investigation suction blister fluid was collected 4, 9 and 24 h after a dose of 6 times the MED for UVR-B.

Dose-response studies

The individual minimal erythematous dose (MED) for UVR-B was determined in each of the group of subjects employed for this investigation. The visual assessment of an apparent papular reaction was also determined. Fresh test sites were subsequently irradiated with 1, 3, 6, and 10 times the MED and with the papular response dose on each subject, and 24 h later suction bullae were raised as described above. At least one unirradiated site was always included as control.

In the cases of UVR-A and visible light single doses only (30 and 60 J.cm⁻² respectively) were employed.

Latency studies

The influence of the interval between irradiation with UVR-B and blister formation was studied using a standard dose of 3 times the MED. In this group of subjects 3-6 sites were irradiated on each individual. Bullae were raised at various times after irradiation, fluid collected and enzyme assays performed as before.

Effect of UVR on blister fluid in vitro

0.5 ml samples of blister fluid were irradiated with UVR-B (30, 180 or 600 mJ.cm⁻²), UVR-A (16, 25 or 59 J.cm⁻²) or visible light (23, 70 or 140 J.cm⁻²). During the irradiation samples were shaken continuously and dose-rates adjusted so that the temperature of the samples did not rise appreciably. After irradiation the samples (together with unirradiated controls) were diluted 1:3 with water and enzyme assay carried out as before.

RESULTS

General

The MED for UVR-B varied between 80 and 200 mJ.cm⁻². Erythema at this dose appeared 6-8 h after exposure.

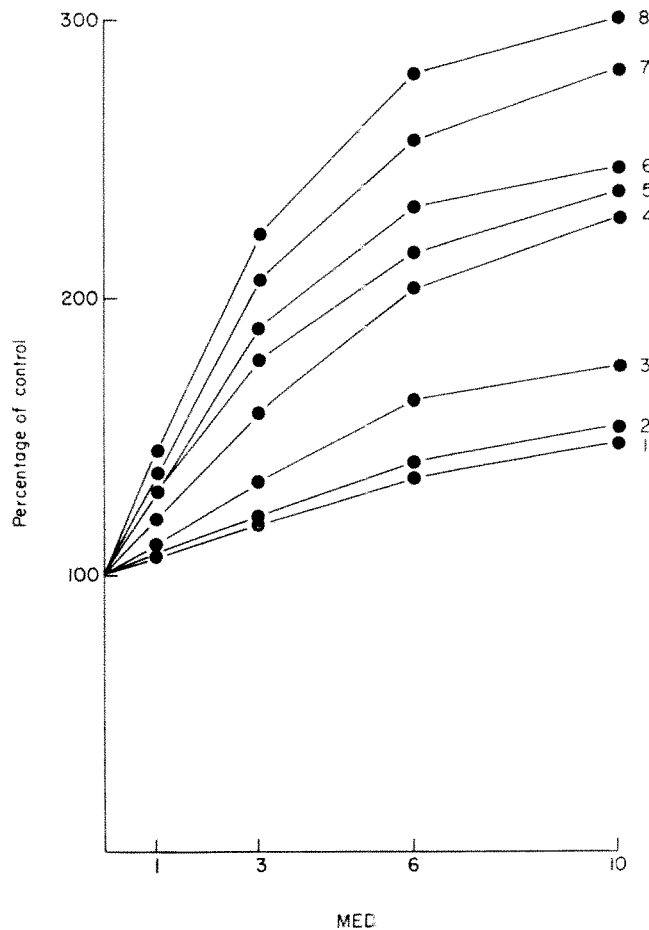


FIGURE 1. Mean acid hydrolase values of 4-7 bullae 24 h after irradiation with UVR-B (filters KG₁+310). Percentage elevation with increased radiation dose is compared with bullae of non-irradiated skin for: (1) β -glucuronidase; (2) β -acetylglucosaminidase; (3) arylamidase; (4) acid phosphatase; (5) α -galactosidase; (6) α -glucosidase; (7) α -mannosidase; (8) β -galactosidase.

The suction pressure required to raise a bulla on the skin of the abdomen was about 200-250 mmHg. This is rather less than that used on the forearms (300-400 mmHg). The suction time for non-irradiated abdominal skin was about the same as that for the forearm; however, erythematous skin required an average of 15 min less and skin showing a papular response required about 25 min less suction time than non-irradiated skin.

No significant increase was observed in protein concentration or in cellular content of the suction blister fluid 4, 9 and 24 h after a preceding dose of 6 times the MED for UVR-B.

Dose-response curve: UVR-B

The activity of all enzymes in suction blisters, raised 24 h after irradiation, was increased even using a dose of 1 MED. At higher doses (3-10 MED) the percentage increase in enzyme activity over the control was in most cases approximately proportional to the magnitude of the dose (Fig. 1). About the same percentage increment was seen after the papular reaction as after a dose of approximately

TABLE 2. Mean values and range (in parentheses) of hydrolase activity in blister fluid from 7 bullae (BF; dose 10 MED, latent period 24 h), in normal human epidermis (Mier & v.d.Hurk, 1976) and in leukocytes (Mier & v.d.Hurk, personal communication)

Enzyme	BF control (pmol/min/ml)		BF irradi. (pmol/min/ml)		Increment in BF (pmol/min/ml)	Epidermis (pmol/ min/mg)	Leukocytes (pmol/min/ mg prot.)
	Mean value	Range	Mean value	Range			
β -glucuronidase	1800	(1400-2300)	2700	(2100-3500)	900	50	1800
β -acetylglucosaminidase	4300	(3400-5500)	6600	(5200-8400)	2300	130	2100
arylamidase	150	(120-170)	260	(210-310)	110	300	3000
acid phosphatase	5700	(4000-7100)	13,000	(8400-17000)	7300	880	2100
α -galactosidase	29	(11-49)	69	(26-110)	40	10	150
α -glucosidase	62	(45-83)	150	(90-200)	88	11	370
α -mannosidase	100	(60-150)	280	(190-400)	180	28	270
β -galactosidase	120	(70-160)	360	(220-530)	240	42	1300

TABLE 3. Mean values and range (in parentheses) of hydrolase activity from 7 unirradiated bullae (same as Table 2), bullae from 6 erythematous reactions=1 MED and bullae from 6 papular reactions 24 h after irradiation with UVR-B (filters KG₁+310). All values are expressed as pmol/min/ml

Enzyme	BF control		BF erythematous reaction		BF papular reaction		Increment	
							eryth. react.	pap. react.
β -glucuronidase	1800	(1400-2300)	1900	(1600-2500)	2300	(1900-2900)	100	500
β -acetylglucosaminidase	4300	(3400-5500)	4600	(3700-5900)	5600	(4600-6800)	300	1300
arylamidase	150	(120-170)	170	(150-190)	210	(170-260)	20	60
acid phosphatase	5700	(4000-7100)	6600	(5300-8000)	9500	(7800-11100)	900	3800
α -galactosidase	29	(11-49)	38	(23-61)	57	(38-80)	9	28
α -glucosidase	62	(45-83)	81	(56-101)	130	(85-170)	19	68
α -mannosidase	100	(60-150)	130	(100-170)	210	(160-260)	36	110
β -galactosidase	120	(70-160)	170	(120-200)	250	(200-320)	50	130

4-5 times the MED (Table 3). Although the largest percentage increases were found in the glycoside hydrolases β -galactosidase and α -mannosidase, the greatest increments in absolute terms (calculated for 10 MED, Table 2, col. 4) were seen in acid phosphatase and β -acetylglucosaminidase.

Response to UVR-A and visible light in vivo

For UVR-A a very faint and barely visible erythema was observed 8 h or more after exposure in a few cases, although irradiation time was more than $\frac{1}{2}$ h. There was no significant change in the levels of enzyme activity in blister fluid from these reactions. Neither an erythematous skin reaction nor any significant change in the levels of enzyme activity in the blister fluid was observed following visible light after doses of 60 J.cm⁻².

Latency studies

The results are shown in Figs 2 and 3; again all values have been expressed as a percentage of the

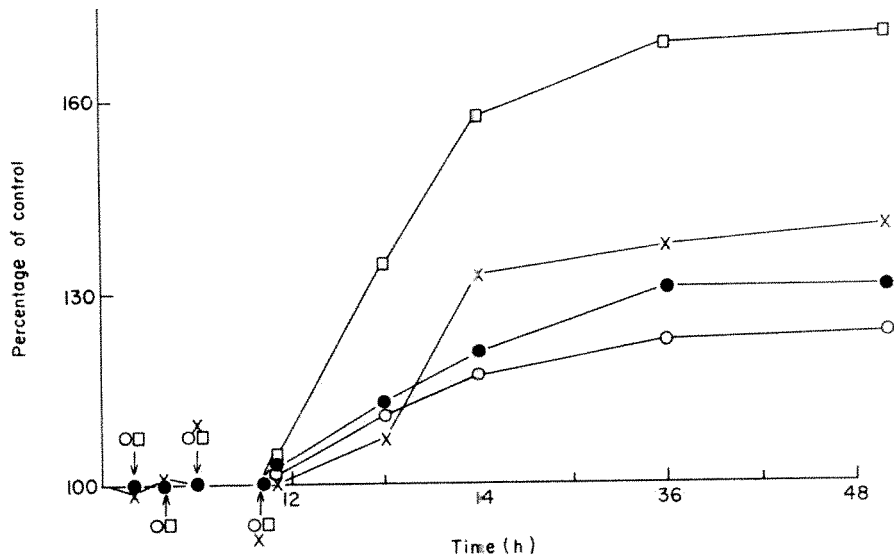


FIGURE 2. Mean acid hydrolase values of 3-6 bullae at 2, 4, 6, 10, 11, 18, 24, 36 and 48 h after irradiation with 3 MED (UVR-B) expressed as percentage of control. (○) β -glucuronidase; (●) β -acetylglucosaminidase; (×) arylamidase; (□) acid phosphatase.

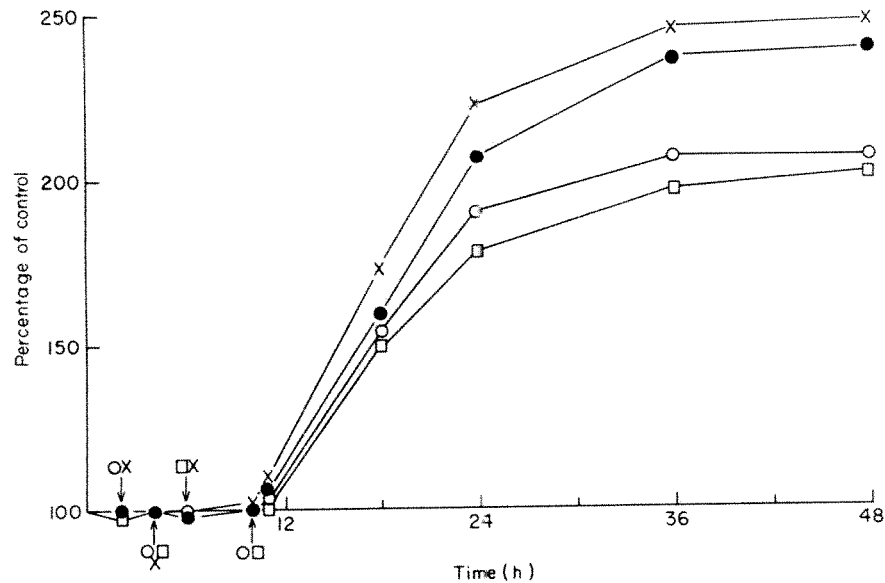


FIGURE 3. As Fig. 2 for (□) α -galactosidase; (○) α -glucosidase; (●) α -mannosidase and (×) β -galactosidase.

unirradiated control. In certain cases small increases in activity were observed 11 h after irradiation, but in general these did not reach significant levels until 18 h post irradiation. Activity continued to rise until 48 h; this was the longest interval investigated.

TABLE 4. Effect of UV irradiation on acid hydrolases of suction blister fluid *in vitro*. Activity expressed as percentage of control (100%)

Enzyme	Irradiation dose with KG ₁ +310 filters			Irradiation dose with KG ₁ +WG ₃₂₀ +UG ₅ filters			Irradiation dose with KG ₁ +GG ₄₉₅ filters		
	30 mJ.cm ⁻²	180 mJ.cm ⁻²	600 mJ.cm ⁻²	16 J.cm ⁻²	25 J.cm ⁻²	59 J.cm ⁻²	23 J.cm ⁻²	70 J.cm ⁻²	140 J.cm ⁻²
Acid phosphatase	90	85	81	82	71	62	97	90	86
α -glucosidase	100	99	98	99	95	93	99	97	97
α -galactosidase	97	99	97	76	75	70	98	96	94
β -galactosidase	87	81	75	62	55	50	72	61	61
α -mannosidase	93	90	86	58	49	45	90	88	83
β -acetylglucosaminidase	98	95	95	94	90	87	97	100	96
β -glucuronidase	96	96	92	100	100	98	98	101	101
Arylamidase	98	98	96	97	99	97	101	100	98

Effect of UVR in vitro

The effects of UVR-A, UVR-B and of visible light on the enzymes *in vitro* are shown in Table 4. In general these changes are relatively small; marked decreases in activity were seen only in the cases of β -galactosidase and α -mannosidase. The apparently greater effect of UVR-A (compared with UVR-B) is clearly the result of the much greater doses employed, and does not indicate any specific photochemical inactivation in this region.

DISCUSSION

A number of workers have attempted to demonstrate lysosomal damage following exposure of skin to UVR. Several different approaches have been employed, each with serious limitations. The measurement of total hydrolase activity after irradiation (Block & Anglin, 1967; Anglin & Burns, 1968; Johnson, 1968) rests on the unproven assumption that 'free' hydrolases will diffuse rapidly out of the tissue, and also ignores changes in the rate of *de novo* enzyme synthesis. Histochemical observation at the e.m. level has posed the usual problems of lack of specificity and difficulty of quantification, although certain recent work (Hönigsmann, Wolff & Konrad, 1974) provides at least a strong indication for lysosomal damage at a late stage of the UVE reaction. Finally, the perfusion of skin *in vivo* (Greaves & Søndergaard, 1970) results in an unacceptably high dilution of the enzymatic activity.

The approach described here, namely the use of the experimental suction blister as a means of recovering diffusible mediators (Black *et al.*, 1977), seems to overcome these limitations. The present results demonstrate that large, dose-related increases in the levels of acid hydrolase activity in blister fluid do occur as a late consequence of irradiation with UVR-B. By contrast, no increases were seen in skin irradiated with UVR-A or visible light. Since the possibility of direct photochemical activation has been ruled out by our experiments *in vitro* (Table 4), we must conclude that the increased activity following irradiation can only represent leakage into the bulla from an external source.

Before claiming this source to be the epidermis, three other possibilities must be ruled out. Firstly, it is hardly likely that the increased activity can be derived via interstitial fluid from the plasma, since the enzyme concentrations in the bullae (even before irradiation) are higher than those of interstitial fluid (Volden, 1978a-c). Second, the dermal fibroblasts are an equally unlikely source because

of their relative paucity and because of the very small dose of UVR-B which penetrates into the dermis.

The third possibility is diffusion from invading leukocytes; this seems particularly inviting because the increases occur only at a late stage in the reaction (Figs 1 & 2). Fortunately the 'profiles' of hydrolase activity have been established both for normal human epidermis (Mier & v.d.Hurk, 1976) and for leukocytes (Mier & v.d.Hurk, personal communication); these may be compared with the pattern of increases found in blister fluid (Table 2). Statistical analysis of these values shows, with the exception of arylamidase (see below), an extremely good correlation between the changes in blister fluid and the hydrolase profile of epidermis ($r=0.98$); the correlation with the hydrolase profile of leukocytes is relatively poor ($r=0.67$). Although some contribution from inflammatory cells is obviously not excluded, therefore, it seems certain that the epidermal lysosome is at least the major source of the increased hydrolase activity seen in blister fluid following exposure to UVR.

An interesting and unexpected finding arising from the data in Table 2 is that the enzyme (or group of enzymes) termed 'arylamidase', although extremely active in both epidermis and in leukocytes, shows relatively little increase in blister fluid. The most likely interpretation of this observation is that epidermal arylamidase is not a lysosomal enzyme. Mahadavan & Tappel (1967) have shown it to occur in both lysosomes and microsomes of liver and kidney; its status in other tissues has remained doubtful.

In summary, then, we may claim to have provided strong evidence that the lysosome is indeed involved in the sequence of events following UV irradiation. Nevertheless, the fact that the release of free lysosomal enzymes cannot be demonstrated until 11 h postirradiation (i.e. several hours *after* erythema is visible) makes it clear that lysosomal rupture is not the initial event in the inflammatory pathway. Of the many alternative possibilities, perhaps the most attractive is the plasma membrane as the primary site of interaction with UVR; various subsequent routes can be postulated culminating in lysosomal breakdown. Further investigations will concern patients showing a clinically abnormal response to UVR.

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REFERENCES

- ANGLIN, J.H. & BURNS, P.G. (1968) Changes in epidermal acid phosphatase activity following UV-irradiation. *Journal of investigative Dermatology*, **50**, 355.
- BLACK, A.KOBZA, GREAVES, M.W., HENSBY, C.N., PLUMMER, N.A. & EADY, R.A. (1977) A new method for recovery of exudates from normal and inflamed human skin. *Clinical and Experimental Dermatology*, **2**, 209.
- BLOCK, O. & ANGLIN, J.H. (1967) Ultraviolet light alterations of acid maltase activity in epidermis. *Journal of investigative Dermatology*, **48**, 252.
- DESAI, I.D., SAWANTI, P.L. & TAPPEL, A.L. (1964) Peroxidase and radiation damage to isolated lysosomes. *Biochimica et Biophysica Acta*, **86**, 277.
- GREAVES, M.W. & SØNDERGAARD, J. (1970) Pharmacologic agents released in ultraviolet inflammation studied by continuous skin perfusion. *Journal of Investigative Dermatology*, **54**, 365.
- HÖNIGSMANN, H., WOLFF, K. & KONRAD, K. (1974) Epidermal lysosomes and ultraviolet light. *Journal of Investigative Dermatology*, **63**, 337.
- JOHNSON, B.E. (1968) Ultraviolet radiation and lysosomes in skin. *Nature*, **219**, 1258.
- JOHNSON, B.E. & DANIELS, F. (1969) Lysosomes and the reactions of the skin to ultraviolet irradiation. *Journal of Investigative Dermatology*, **53**, 85.
- LAZARUS, G.S. & HATCHER, V.B. (1975) Lysosomes and the skin. In: *Lysosomes in Biology and Pathology* (Ed. by J.T.Dingle and R.T.Dean), vol. 4, p. 111. North-Holland Publishing Co., Amsterdam.
- MAHADEVAN, S. & TAPPEL, A.L. (1967) Arylamidases of rat liver and kidney. *Journal of Biological Chemistry*, **242**, 2369.

- MIER, P.D. & VAN DEN HURK, J.J.M.A. (1976) Lysosomal hydrolases of the epidermis. 6. Changes in disease. *British Journal of Dermatology*, **95**, 271.
- MIER, P.D. & VAN DEN HURK, J.J.M.A. (1977) Personal communication.
- VOLDEN, G. (1978) Acid hydrolases in blister fluid. 1. Characterization and quantification of acid phosphatase. 2. Characterization and quantification of glycoside hydrolases. 3. Characterization and quantification of peptide hydrolases. *British Journal of Dermatology*, **98**, 273, 281 & **99**, 49.
- VOLDEN, G. & THUNE, P.O. (1977) Light sensitivity in mycosis fungoides. *British Journal of Dermatology*, **97**, 279.
- WEISSMAN, G. & DINGLE, J. (1961) Release of lysosomal protease by ultraviolet irradiation and inhibition by hydrocortisone. *Experimental Cell Research*, **25**, 207.
- WEISSMAN, G. & FELL, H.B. (1962) The effect of hydrocortisone on the response of fetal rat skin in culture to ultraviolet irradiation. *Experimental Cell Research*, **116**, 305.

Mycosis fungoides, nitrogen mustard and skin cancer

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SUMMARY

The prevalence of epithelial cancer was determined in 202 patients with mycosis fungoides and the Sézary syndrome and was found to be 10.5%. In an attempt to establish a possible association with topically applied nitrogen mustard, it was noted that 4.0% of these patients had lesions prior to nitrogen mustard therapy (although over half subsequently developed further lesions) and 6.5% of the patients developed them *de novo* after therapy. In two patients in this latter group, malignancies developed in areas not usually associated with solar-induced cancer. These findings indicate that epidermal neoplasia is not uncommon in these patients and that nitrogen mustard may function as a carcinogen or a co-carcinogen.

Nitrogen mustard (mechlorethamine, HN_2), a radiomimetic agent, is a known carcinogen inducing lung tumours in mice when given systemically (Heston, 1949). Preliminary studies on the application of HN_2 to hairless mouse skin suggest that it may be a cutaneous carcinogen (Davies, R., personal communication, 1977). It was decided to investigate whether patients with mycosis fungoides (MF) and its Sézary variant are prone to develop epithelial malignancies, either prior to or after being treated with topically applied HN_2 .

METHOD

The records of 202 patients (135 males and 67 females) with mycosis fungoides and its Sézary variant, treated with topically applied HN_2 and followed for intervals of up to 9 years, were reviewed to assess the prevalence of epithelial malignancy. Factors taken into account were age, sex, race, stage of mycosis fungoides (Van Scott & Kalmanson, 1973), other treatment modalities and the presence of actinic damage noted prior to initiating treatment.

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RESULTS

Twenty-four of 202 patients with mycosis fungoides or the Sézary syndrome had developed epithelial cancers by the time of this review. Three patients had previously received arsenic for therapy and were excluded from the study. Thus the circumstances concerning the neoplasms in the remaining 21 patients (10.5%) were analysed in detail. There were 12 males and 9 females. All were Caucasians and the mean age at onset of lesions was 64 ± 12 (s.d.) years. The mean time following the initiation of HN₂ therapy until the detection of epithelial neoplasms other than actinic keratoses was 4.0 ± 1.9 (s.d.) years.

Eight patients (4%) had lesions removed prior to starting nitrogen mustard therapy. (Four had actinic keratoses and four had basal cell carcinomas.) After starting treatment, five of these eight patients developed additional lesions. These are listed as Group I in Table 1. The remaining thirteen patients (6.5%) had no evidence of epithelial lesions initially, but subsequently acquired lesions. Four patients developed actinic keratoses only, three patients had actinic keratoses plus additional lesions and six had neoplasms without associated actinic keratoses. Details of these except for the four patients with actinic keratoses are, in Table 1, designated as Group II. The patients are thus divided into those with (Group I) and those without preceding evidence of skin neoplasia (Group II).

The prevalence of tumours developing after HN₂ therapy is as follows: squamous cell carcinomas (SCC) 5.0%, keratoacanthomas (KA), 3.0%, and basal cell carcinomas (BCC) 1.5%. Since there were also 4 basal cell carcinomas which were removed prior to HN₂ therapy the prevalence of these tumours in patients with mycosis fungoides irrespective of whether they developed before or after treatment is 3.5%.

Two lesions occurred in non sun-exposed areas in patients who had no evidence of skin cancer prior to starting HN₂ treatment. One was a squamous cell carcinoma on the scrotum appearing 6

TABLE 1. Epithelial neoplasms other than actinic keratoses occurring in patients with mycosis fungoides after treatment with HN₂

Patients			Tumour types and detection time (years) after HN ₂	Other therapies
Age	Sex	Disease stage		
Group I				
73	M	I	SCC(6), SCC(6)	Electron beam
70	M	II	BCC(4), SCC(3), KA(4)	
71	M	II	KA(3), KA(6)	
45	M	Sézary	SCC(5)	MTX, Bleomycin
68	F	Sézary	KA(1)	
Group II				
61	F	I	SCC(7)	MTX BCNU MTX MTX Chlorambucil MTX, Chlorambucil
50	F	II	KA(7)	
66	M	II	BCC(6)	
71	M	II	SCC(3)	
71	M	III	SCC(6), KA(3)	
82	F	III	BCC(1), SCC(1)	
75	M	IV	SCC(4)	
70	M	Sézary	SCC(4)	
83	F	Sézary	KA(5), SCC(2)	

MTX = methotrexate; BCNU = bis(2-chlorethyl)-1-nitrosourea.

years later. It was estimated that the patient had applied approximately 14 g of HN_2 over 4 years of therapy. The other lesion was a squamous cell carcinoma which developed in a pre-existing epidermal naevus on the buttock of a female 7 years after commencing therapy. She had applied approximately 16 g of HN_2 .

As recorded in Table 1, a number of patients received other treatment modalities prior to the onset of the epithelial malignancies, but only one patient had received a potential skin carcinogen, whole body electron beam therapy 10 years before developing 2 squamous cell carcinomas on the front of the chest.

DISCUSSION

The most convincing evidence that HN_2 applied topically is a carcinogen or co-carcinogen is the finding of two squamous cell carcinomas in locations protected from solar irradiation. The appearance of epithelial malignancies in non sun-exposed areas usually suggests exposure to benzpyrene or a related chemical, ionizing irradiation (Traenkle, 1963) or arsenic ingestion (Sommers & McManus, 1953). The two patients in this series had not been exposed to any of these modalities and it becomes highly suggestive that HN_2 is responsible.

The overall prevalence for basal and squamous cell carcinomas in this study of mycosis fungoides, including those patients who developed carcinomas prior to HN_2 therapy, was 7.5% (5% male and 2.5% female). The prevalence of carcinomas which developed after starting HN_2 therapy was 5.5% (4% male and 1.5% female).

The incidence of basal and squamous cell carcinomas in Dallas, Texas, where exposure to damaging ultraviolet light irradiation is very high, is 3.2% per year for males and 1.6% per year for females for the 65-75 age group (Scotto, Kopf & Urbach, 1974). In County Galway, Ireland, where a relatively homogeneous population has a cutaneous genetic predisposition to developing epithelial malignancy, the prevalence of basal and squamous cell carcinomas of the skin for the 65 age group is 5.0% for males and 3.0% for females (O'Beirn *et al.*, 1968).

These observations on nitrogen mustard are preliminary. Data from a control population gathered locally would not have been meaningful since the patients with M.F. are referred from a wide area and although direct comparison with the Dallas and Galway studies is not possible the prevalence rates for basal and squamous cell carcinomas in this study are comparably high.

It is the current belief that mycosis fungoides must be treated as early as possible when it is limited to the skin to effect remission and cure of this potentially fatal disease. HN_2 therapy and electron beam irradiation (Fuks, Bagshaw & Farber, 1973) are the only established approaches at present available to this end. The epithelial malignancies observed in this study were easily treated and it cannot be concluded at this stage that topically applied HN_2 is an inappropriate treatment for mycosis fungoides.

REFERENCES

- FUKS, Z.Y., BAGSHAW, M.A. & FARBER, E.M. (1973) Prognostic signs and the management of the mycosis fungoides. *Cancer*, **32**, 1385.
- HESTON, W.E. (1949) Carcinogenic action of the mustards. *Journal of the National Cancer Institute*, **10**, 125.
- O'BEIRN, S.F., JUDGE, P., URBACH, F., MACCON, C.F. & MARTIN, F. (1968) Skin cancer in County Galway, Ireland. *Sixth National Cancer Conference Proceedings*, p. 489. Lippincott, Philadelphia & Toronto.
- SCOTTO, J., KOPF, A.W. & URBACH, F. (1974) Non melanoma skin cancer among Caucasians in four areas of the United States. *Cancer*, **34**, 1333.
- SOMMERS, S.C. & MCMANUS, R.G. (1953) Multiple arsenical cancers of skin and internal organs. *Cancer*, **6**, 347.
- TRAENKLE, H.L. (1963) X-ray induced skin cancer in man. *National Cancer Institute Monograph*, **10**, 423.
- VAN SCOTT, E.J. & KALMANSON, J.D. (1973) Complete remissions of mycosis fungoides lymphoma induced by topical nitrogen mustard (HN_2). Control of delayed hypersensitivity to HN_2 by desensitization and by induction of specific immunologic tolerance. *Cancer*, **32**, 18.

Biological changes of human cutaneous nerves caused by ultraviolet irradiation: an ultrastructural study

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SUMMARY

Three white male volunteers were irradiated by long wave ultraviolet (UV-A) and by solar simulating radiation (SSR). An acute change of cutaneous nerves was found following a single exposure of UV-A irradiation. Non-myelinated Schwann cells and perineural cells in the papillary and reticular dermis were degenerated. Axoplasms appeared to be electron-dense, but were less affected than Schwann cells. After long-term repeated exposure, many free nerve endings were found in the dermo-epidermal junction above the basal lamina and some of them made a terminal enlargement. In one instance an axon made a swelling. Dermal free nerve endings also seemed to be increased in number and some of them were situated immediately beneath the melanocytes which were active in melanogenesis. Multiplication of basal lamina of the Schwann cells and perineural cells was observed. Amorphous material was precipitated around the non-myelinated Schwann cells. After a single exposure of SSR irradiation the degeneration of Schwann cells and axons in the dermo-epidermal junction was less severe than after UV-A and changes were minor in the reticular dermis. After repeated exposure, intra-epidermal proliferation of free nerve endings was detected. Dermal nerves were slightly affected. No intra-epidermal free nerve endings were observed in controls.

The intra-epidermal proliferation of free nerve endings was confirmed following repeated UV exposures. Melanocyte-nerve association is suggested to be the cause of stimulating melanocyte activity.

Intra-epidermal free nerve endings have rarely been observed in normal human epidermis by electron microscopy (Winkelman, 1967; Breathnach, 1971; Chouhkov, 1972; Orfanos & Mahrle, 1973; Cauna, 1973), and they were assumed to be related to Merkel cells (Winkelman & Breathnach, 1973). In some depigmentation disorders, however, the proliferation of free nerve endings in the epidermis

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was observed—in a halo naevus (Hashimoto, 1974), vitiligo vulgaris, incontinentia pigmenti achromians of Ito, and Vogt-Koyanagi syndrome (Morohashi *et al.*, 1977). Some role was ascribed to these intra-epidermal nerve endings, such as melanogenesis, because of the finding of melanocyte-nerve association. Still, it was uncertain whether this phenomenon is related to the cause of melanocyte degradation or to melanocyte regeneration. An increase of nerve fibres was observed at the light microscope level by FitzGerald, Folan & O'Brien (1975) in hairless mice after they received multiple doses of ultraviolet irradiation (280–350 nm). On the other hand, cutaneous nerve alteration at the ultrastructural level, caused by ultraviolet (UV) irradiation, has not been adequately explored; particularly, the effect of UV-A has just begun to be explored (Kumakiri, Hashimoto & Willis, 1977). In UVA-methoxsalen therapy (PUVA) for psoriasis, skin pigmentation is associated with increased intra-epidermal nerve endings (Kumakiri & Hashimoto, 1978) and pruritus (Wolff *et al.*, 1976). The present study was to explore systematically the cutaneous nerve-melanocyte relationship in UV-treated human skin.

MATERIALS AND METHODS

Subjects. Three white male volunteers were examined. The midportions of their backs served as the test sites. The specimens were taken with 2 mm skin punches and cut into about 1 mm³ pieces.

Light sources. Solar simulating radiation (SSR) in the range of 290–420 nm was obtained from a 1600 W xenon solar-simulating lamp source (Berger, 1969). Radiation of 320–400 nm (UV-A) was achieved by adding a Schott WG-345 filter to the above system; this filter gives a transmission of 50% of its peak at 345 nm and cuts off practically all rays below 320 nm (less than 0.01% transmission at 315–320 nm). This will henceforth be called UV-A radiation although a very small amount of near visible energy is also present (<0.022% at 400–420 nm).

Energy measurements were obtained using an Eppley photocell and Keithley 145 millimicro-voltmeter. Special characteristics were obtained using an International Light Spectroradiometric System. Energy in the 320–400 nm range delivered to the surface of a test site was 252 mW/cm².

Single exposures. Two volunteers (nos 1 and 2) were irradiated in separate areas (each area measuring 1 cm²) with UV-A and SSR by solar simulator. Volunteer 1 was irradiated with UV-A (0 and 130 J/cm²) and with SSR (15 J/cm²). Biopsies were taken 15 min and 24 h later and fixed for 4 h in 5% glutaraldehyde in 1/10 M cacodylate buffer adjustment to pH 7.4. Volunteer 2 was irradiated with UV-A (0, 86 and 173 J/cm²) and with SSR (23 J/cm²) and biopsies were taken after 48 h from each area and fixed as described above.

Repeated exposures. UV-A and SSR obtained by solar simulator were administered to each site at approximately 2 week intervals for nearly a year. During this period, volunteer 1 was exposed 20 times, receiving each time 130 J/cm² of UV-A and 15 J/cm² of SSR. The total dose received by volunteer 1 was therefore 2.6×10^3 J/cm² of UV-A and 3.0×10^2 J/cm² of SSR. Volunteer 3 was exposed 17 times, receiving each time 112 J/cm² of UV-A and 13 J/cm² of SSR. Thus, he received 1.9×10^3 J/cm² of UV-A and 2.2×10^2 J/cm² of SSR. The irradiated areas of Volunteer 1 were biopsied 5 days after the last exposure and those of Volunteer 3 were biopsied 15 days after. All specimens were immediately fixed in the same glutaraldehyde. Non-irradiated control specimens were also biopsied and similarly fixed.

Electron microscopy. The tissues fixed in glutaraldehyde were post-fixed in 1% osmium tetroxide in the same buffer as used for glutaraldehyde. Routine methods (Hashimoto, 1974) were used for

embedding, thin sectioning, and staining. Twenty serial sections obtained from each tissue block were observed in an Hitachi HU-11C electron microscope.

Epidermal length (Table 1). The length of epidermis in each block was measured in thick sections (about 1000 nm) stained with Azure B. The measurement was taken in a Zeiss Photomicroscope using a stage micrometer. The entire length of the epidermis thus measured was examined in the thin sections. The areas covered by grid mesh were re-examined in serial sections. To avoid a duplication of observation, only one serial section was used from each block, whenever possible. Three or four blocks from each biopsy were examined.

RESULTS

The general effects of UV irradiation on the skin have already been reported (Kumakiri *et al.*, 1977); therefore, we describe here the details of the cutaneous nerves only. A summary of the data is presented in Table 1.

Single exposure of UV-A. The destructive change was most prominent in the biopsy of 48 h after irradiation and was dose dependent; the change in specimens of 20 min and 24 h was mild and that of 86 J/cm² was less severe than in the 173 J/cm² specimen. Dermal free nerve endings situated below the epidermis showed destruction of Schwann cell cytoplasm (Fig. 1). Axon structure was relatively well preserved; however, the density of axoplasm was increased and the organelles presented degenerative changes. In the reticular dermis, Schwann cells and perineural cells were sometimes completely destroyed (Fig. 2), and basal lamina represented the cell in outline. No free nerve endings were observed in the epidermis of the biopsy after 15 min, 24 h and 48 h.

Single exposure of SSR. The changes of the dermal free nerve endings in the dermo-epidermal junctions were mild and Schwann cell cytoplasm showed less severe changes than those caused by UV-A (Fig. 3). The organelles of axons were affected. In the reticular dermis only minor changes were observed in nerves. No intra-epidermal nerve endings were seen in the epidermis of any specimens.

Repeated (17-20 times) exposures of UV-A. Fourteen intra-epidermal free nerve endings were observed in the epidermis (Table 1). They were above the basal lamina of the epidermis (Figs 4-6). No nerve endings were found in the Malpighian or granular layers. Sometimes terminal bulb formation of these nerves was seen (Fig. 5). They contained a few mitochondria and neurotubules. Many intra-epidermal nerve endings were covered by Schwann cell sheath; however, naked axons were also observed. In one instance, a naked axon was cut longitudinally, and showed a swelling (Fig. 6).

In the dermo-epidermal junction, many free nerve endings were observed and some of them were immediately beneath the melanocytes (Fig. 7). These melanocytes had many premelanosomes and each had a well developed Golgi apparatus. In the reticular dermis, nerve fibres were surrounded by amorphous materials (Fig. 8). Sometimes multiplication of the basal lamina of the Schwann cells was present. Strands of the basal lamina were left around Schwann cells (Fig. 9), and suggested the destruction and disappearance of the perineural cells after repeated exposures.

Repeated (17-20 times) exposures of SSR. Two intra-epidermal free nerve endings were observed. Morphologically, they were similar to those of UV-A irradiated skin epidermis, and situated above the basal lamina of the epidermis. Some dermal free nerve endings were situated under the melanocytes. Only minor changes were observed in the nerves located in the reticular dermis.

TABLE 1. Intra-epidermal nerve endings

Case	A single exposure				Repeated exposures												Control					
	UV-A				SSR				UV-A				SSR				1		2		3	
	1	2	2	48 h	15 min	24 h	48 h	15 min	24 h	48 h	5 days	15 days	5 days	15 days	15 days	15 days						
Time between irradiation and biopsy	15 min	15 min	48 h	48 h	15 min	24 h	48 h	15 min	24 h	48 h	5 days	15 days	5 days	15 days	15 days	15 days						
Total dosage (J/cm ²)	130	130	173	86	15	15	173	15	15	2.3	2.6 × 10 ³	1.9 × 10 ³	3.0 × 10 ²	1.9 × 10 ³	2.2 × 10 ²	2.2 × 10 ²						
Number of intra-epidermal nerve endings	0	0	0	0	0	0	0	0	0	0	2	12	1	12	0	0						
Length of epidermis examined (mm)	3.3	1.7	4.35	2.65	2.6	1.7	2.5	2.6	1.7	2.5	4.1	4.6	2.9	4.6	3.1	3.1	2.1	2.1	3.3	2.6		

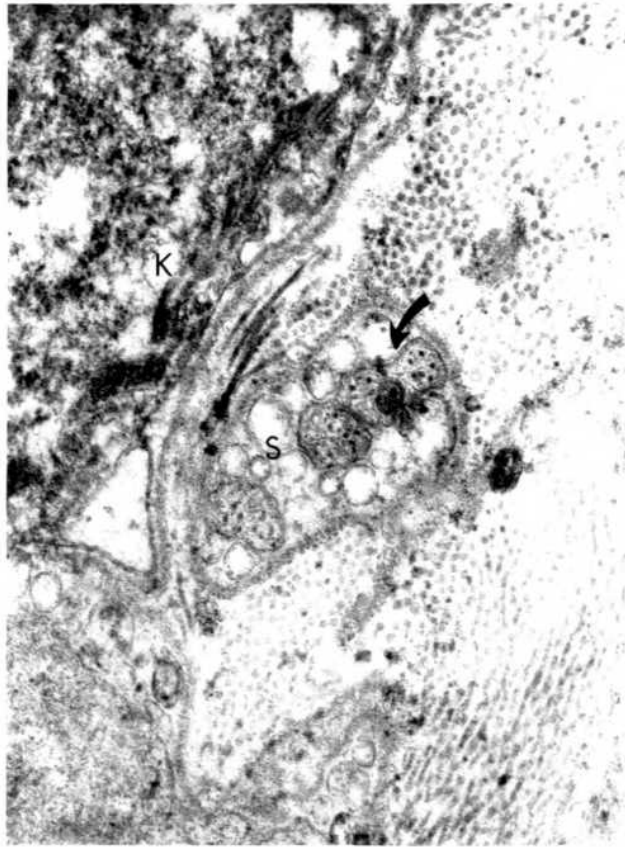


FIGURE 1. The degenerative changes of Schwann (s) cell sheath are seen in the dermo-epidermal junction. The axon (arrow) is pressed by the cell debris. Biopsy was taken 48 h after a single exposure of UV-A. K, keratinocyte ($\times 40,000$).

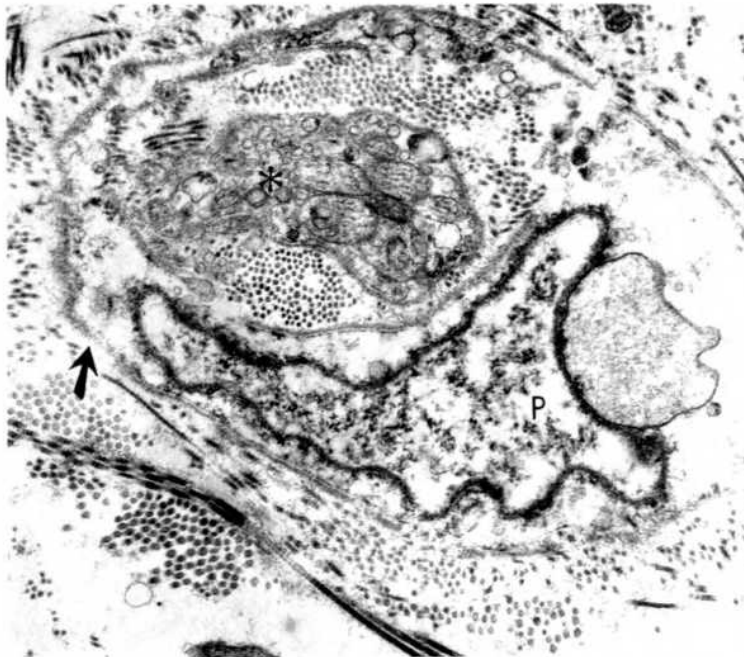


FIGURE 2. Perineural (P) cell and Schwann (*) cell in the reticular dermis are destroyed and basal lamina (arrow) suggests the outline of the cytoplasm. Biopsy was taken 48 h after a single exposure to UV-A ($\times 19,000$).

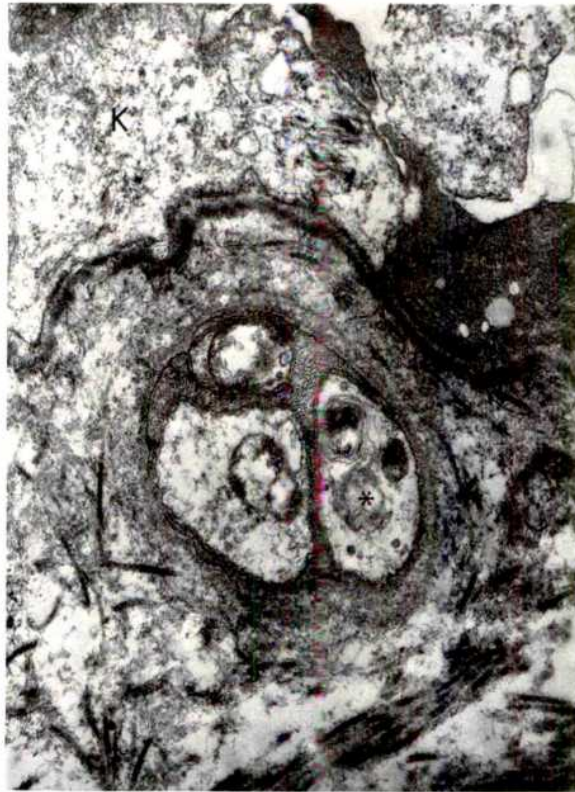


FIGURE 3. Organelles of axoplasm (*) of a subepidermal nerve ending appear to be degenerated. However, the degenerative changes in Schwann cell sheath and axons are less severe than those caused by UV-A (of Fig. 1). Cytoplasm of the basal cell (K) is oedematous. Biopsy was taken 48 h after a single exposure to SSR ($\times 27,000$).

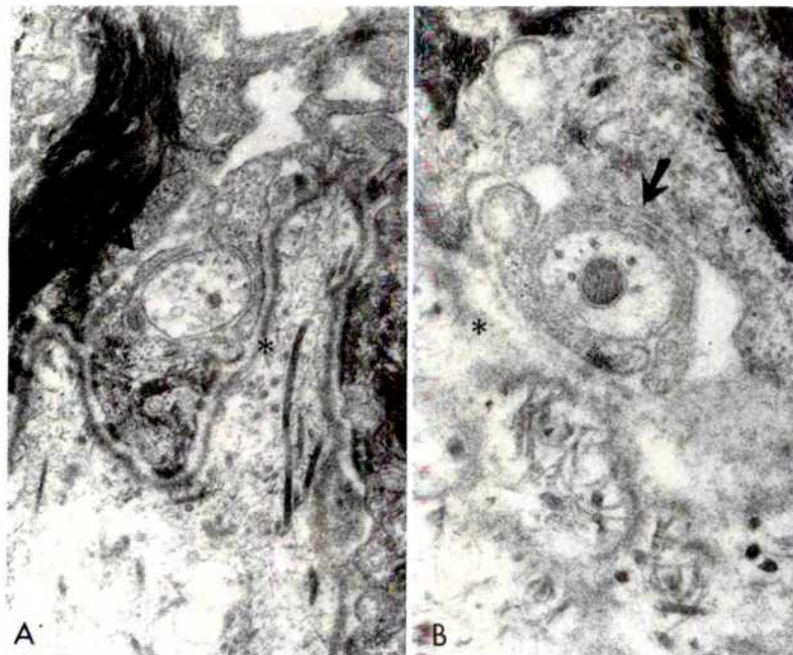


FIGURE 4 (A, B). Intra-epidermal nerve endings (arrows) are seen in the dermo-epidermal junction above the basal lamina (*) of the epidermis. Biopsy was taken after repeated exposures of UV-A. (A) ($\times 18,000$). (B) ($\times 30,000$).

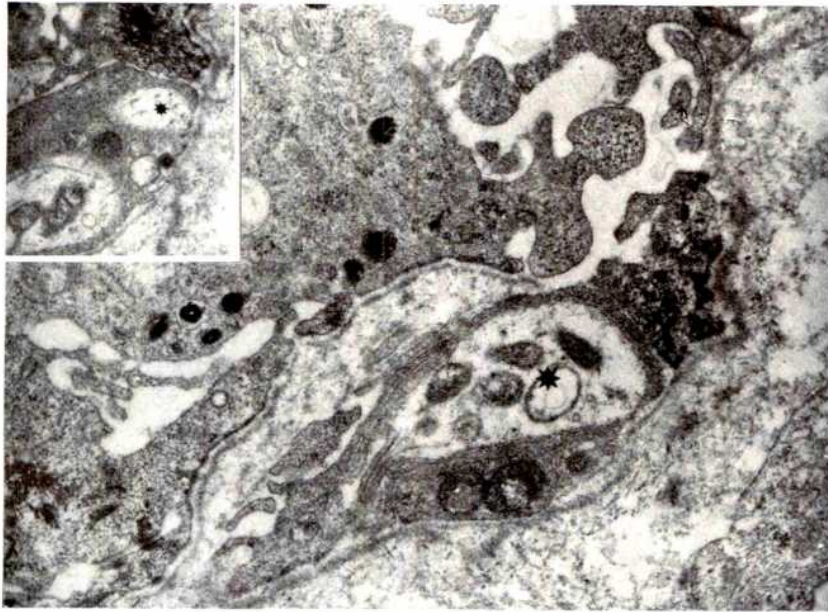


FIGURE 5. Schwann cell-covered axon (*) makes a bulbous enlargement at the dermo-epidermal junction. Biopsy was taken after repeated exposures of UV-A ($\times 24,000$). Insert: A step section to that shown in the main illustration shows several neurotubules in the enlargement ($\times 19,000$).

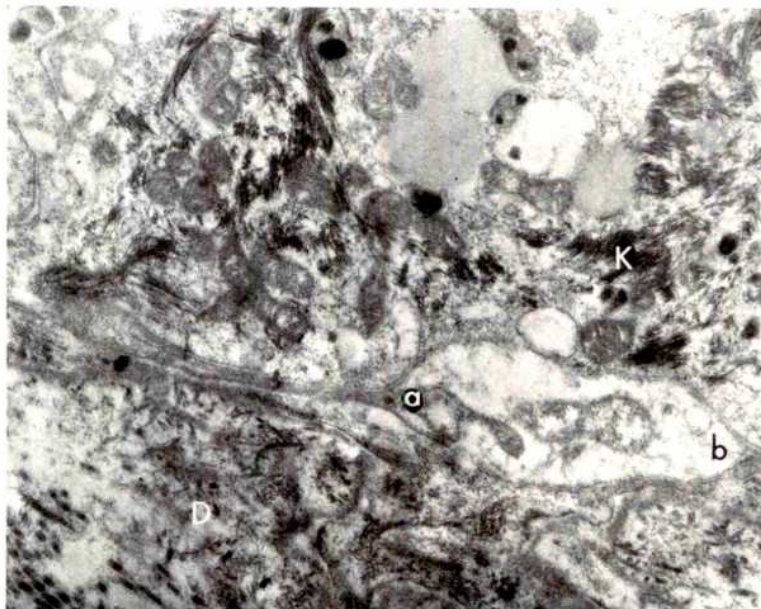


FIGURE 6. A naked axon is surrounded by keratinocytes (K) and is greatly enlarged from (a) to (b). It is suggested that intra-epidermal nerve endings make a swelling. Biopsy was taken after repeated exposures of UV-A. D: Dermis ($\times 22,000$).

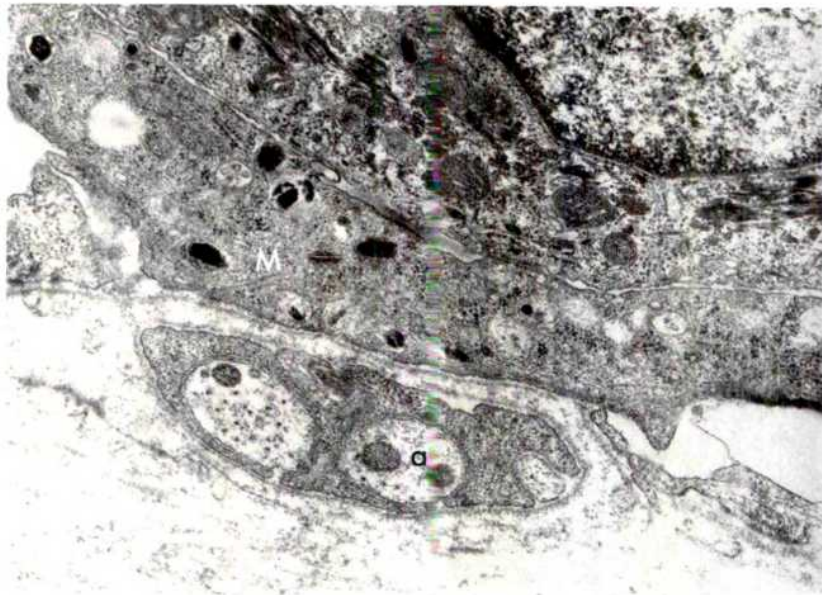


FIGURE 7. Polyaxonal (a) dermal nerve endings are situated directly below the melanocyte (M). Biopsy was taken after repeated exposures of UV-A ($\times 24,000$).

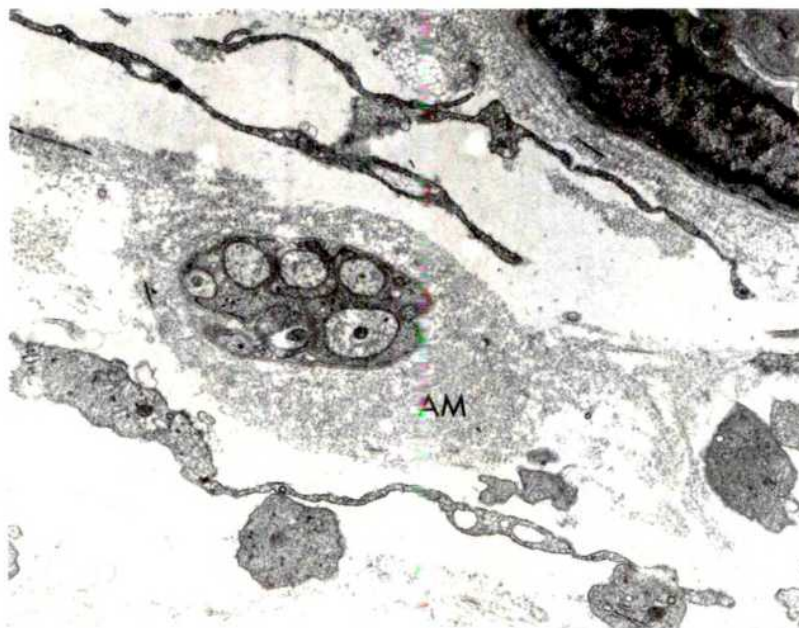


FIGURE 8. In the reticular dermis, a nerve ending is surrounded by an increased amount of amorphous mass (AM). Biopsy was taken after repeated exposures of UV-A ($\times 11,000$).



FIGURE 9. Long strands of the basal lamina (arrow) surround two Schwann cells (S), which appear nearly normal. It is suggested that perineurial cells have disappeared and their basal laminae remained after repeated damage. Biopsy was taken after repeated exposures of UV-A ($\times 9400$).

Control tissues. No nerve endings were detected in the epidermis, and no abnormalities were detected in the dermal nerves. Sometimes nerve endings were situated under the melanocyte; however, none of these were close enough to the melanocyte to be considered significantly related.

DISCUSSION

No nerve endings were detected in the untreated normal epidermis. This is compatible with the findings of previous workers: only a few or no nerve endings have been reported in normal skin (Winkelmann, 1967; Breathnach, 1971; Chouhkov, 1972; Orfanos *et al.*, 1973; Cauna, 1973). On the other hand, the proliferation of intra-epidermal free nerve endings after repeated UV exposures was confirmed at the ultrastructural level. The incidence of nerve endings in the UV-irradiated epidermis was about 1 per 1 mm length. Axons in the epidermis sometimes formed a terminal enlargement (Fig. 5) and some of them were denuded as in animal epidermis (Hay, 1969; Cauna, 1969; Tsuji, 1971). If sections were cut exactly longitudinal to the naked axon, it gave the appearance of a swelling (Fig. 6).

Using the electron microscope, an increase of intra-epidermal nerve endings has been reported in depigmentation disorders. Thus, halo naevus (Hashimoto, 1974), vitiligo vulgaris, incontinentia pigmenti achromians of Ito, and Vogt-Koyanagi syndrome (Morohashi *et al.*, 1977) were shown to have many intra-epidermal nerves in the lesion. Some role was ascribed to these nerve endings because of melanocyte-nerve association. In this study it was observed that dermal nerve endings have a special affinity to some melanocytes which were in active melanogenesis. The melanocytes which were directly in contact with nerve endings had many premelanosomes and well developed Golgi apparatus. Recently, a similar observation was made in the pigmented psoriatic lesion following long-term PUVA therapy (Hashimoto *et al.*, 1978).

Although melanocytes are of neural crest origin, and in lower animals such as *Fundulus heteroclitus* L. (Bikle, Tilney & Porter, 1966) the nerve endings have been found adjacent to and in synaptic junction with the melanophore surface, melanocyte-nerve contact seems to be very rare in mammals (Winkelmann, 1967; Tsuji, 1971). Melanocyte-nerve association observed in this study and in depigmentary conditions, therefore, suggests that the peripheral nerve extends into the epidermis in order to stimulate melanogenesis or melanocyte regeneration. Melanocytes may be under neural control even in the normal situation; such a relationship may become more manifest and visible after proliferative or regenerative stimuli to the melanocytes.

The observation of nerve proliferation was almost consistent with the light microscopic work of FitzGerald *et al.* (1975). They observed an increase of nerve fibres in the hyperplastic, regenerative epidermis of hairless mice after multiple exposures of UV radiation (280–350 nm), and suggested that the increase might occur 24 h after a single exposure. However, we could not find in the human skin any intra-epidermal nerve endings in the 15 min, 24 h and 48 h specimens, but only the degenerative changes of dermal nerves.

The acute effect of the irradiation was stronger and deeper in UV-A irradiated skin than in SSR irradiated skin at the dosage employed in this investigation. Destruction of Schwann cells and perineural cells was characteristic; thus, it was unlike Wallerian degeneration (O'Daly & Imaeda, 1967), in which axonal change is dominant. This destructive effect of UV-A was similar to that on blood vessels in our previous report (Kumakiri *et al.*, 1977). Following repeated exposure to UV-A, amorphous materials developed surrounding the nerve fibres in the reticular dermis. Since multiplication of basal lamina may suggest degeneration of the cells at the site of injury in the nervous system (Vracko, 1974), cutaneous nerves were probably damaged by UV-A and then regenerated. Since Schwann cell cytoplasm and axoplasm seemed to be intact in the specimens biopsied after repeated irradiations, production of amorphous substance seems to protect the nerves from repeated UV-A exposures. It was also confirmed in blood vessels (Kumakiri *et al.*, 1977).

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REFERENCES

- BERGER, D.S. (1969) Specification and design of solar ultraviolet simulators. *Journal of Investigative Dermatology*, **53**, 192.
- BIKLE, D., TILNEY, L.G. & PORTER, K.R. (1966) Microtubules and pigment migration in the melanophores of *Fundulus heteroclitus* L. *Protoplasma*, **61**, 332.
- BREATHNACH, A.S. (1971) *An Atlas of the Ultrastructure of Human Skin*, p. 189. J. & A. Churchill, London.
- CAUNA, N. (1969) The fine morphology of the sensory receptor organs in the auricle of the rat. *Journal of Comparative Neurology*, **136**, 81.
- CAUNA, N. (1973) The free penicillate nerve endings of the human hairy skin. *Journal of Anatomy*, **115**, 277.
- CHOCHKOV, C.N. (1972) On the fine structure of free nerve endings in human digital skin, oral cavity and rectum. *Zeitschrift für Mikroskopisch-Anatomische Forschung*, **86**, 273.
- FITZGERALD, M.J.T., FOLAN, J.C. & O'BRIEN, T.M. (1975) The innervation of hyperplastic epidermis in the mouse: a light microscopic study. *Journal of Investigative Dermatology*, **64**, 169.
- HASHIMOTO, K. (1974) Ultrastructural studies of halo nevus. *Cancer*, **34**, 1953.
- HASHIMOTO, K., KOHDA, H., KUMAKIRI, M., BLENDER, S.L. & WILLIS, I. (1978) Ultrastructural changes in a psoralen-UVA treated psoriatic lesion. *Archives of Dermatology*, in press.

- HAY, E.D. (1960) The fine structure of nerves in the epidermis of regenerating salamander limbs. *Experimental Cell Research*, **19**, 290.
- KUMAKIRI, M., HASHIMOTO, K. & WILLIS, I. (1977) Biological changes due to long wave ultraviolet irradiation on human skin: an ultrastructural study. *Journal of Investigative Dermatology*, **69**, 392.
- KUMAKIRI, M. & HASHIMOTO, K. (1978) Cutaneous nerve stimulation by psoralen-ultraviolet A therapy: an ultrastructural study. *Journal of Investigative Dermatology*, in press.
- MOROHASHI, M., HASHIMOTO, K., GOODMAN, T.F., NEWTON, D.E. & RIST, T. (1977) Ultrastructural studies of vitiligo, Vogt-Koyanagi syndrome and incontinentia pigmenti achromians. *Archives of Dermatology*, **113**, 755.
- O'DALY, J.A. & IMAEDA, T. (1967) Electron microscopic study of Wallerian degeneration in cutaneous nerves caused by mechanical injury. *Laboratory Investigation*, **17**, 744.
- ORFANOS, C.E. & MAHRLE, G. (1973) Ultrastructure and cytochemistry of human cutaneous nerves. *Journal of Investigative Dermatology*, **61**, 108.
- TSUJI, T. (1971) Free nerve endings of the epidermis in hairy and hairless mice. *Journal of Investigative Dermatology*, **57**, 247.
- VRACKO, R. (1974) Basal lamina scaffold-anatomy and significance for maintenance of orderly tissue structure. A review. *American Journal of Pathology*, **77**, 314.
- WINKELMANN, R.K. (1967) Cutaneous nerves. In: *Ultrastructure of Normal and Abnormal Skin* (Ed. by A.S. Zelikson), p. 202. Lea and Febiger, Philadelphia.
- WINKELMANN, R.K. & BREATHNACH, A.S. (1963) The Merkel cell. *Journal of Investigative Dermatology*, **60**, 2.
- WOLFF, K., FITZPATRICK, T.B., PARRISH, J.A., GSCHNAIT, F., GILCHREST, B., HÖNIGSMANN, H., PATHAK, M.A. & TANNEBAUM, L. (1976) Photochemotherapy for psoriasis with orally administered methoxsalen. *Archives of Dermatology*, **112**, 943.

Oral photochemotherapy in the treatment of lichen planus (LP)

CLINICAL RESULTS, HISTOLOGICAL AND ULTRASTRUCTURAL OBSERVATIONS

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SUMMARY

Seven patients with diffuse lichen planus were treated with oral photochemotherapy. Remission was effected in 6 cases. The only failure may be attributable to a low UVA dose. Histological examination after treatment showed disappearance of the superficial dermal lymphocytic infiltrate. Ultrastructural study revealed nuclear alterations of keratinocytes and nuclear and cytoplasmic changes in superficial dermal cells.

Various theories concerning the mode of action of photochemotherapy in lichen planus are discussed in the light of these observations.

The clinical and histological features of lichen planus (LP) are well known although the pathogenesis is obscure.

Current modes of treatment of LP are not always very effective. Oral photochemotherapy is effective in the treatment of mycosis fungoides (Gilchrest *et al.*, 1976) which is characterized by proliferation of T lymphocytes in the skin. With treatment, the cellular infiltrate disappears for ill-defined reasons (Hjorstshøj & Schmidt, 1977; Roenigk, 1977). As the infiltrate of LP is almost entirely composed of T lymphocytes (Alario *et al.*, 1978; Tan, Byron & Hayes, 1975; Walker, 1976; Shousha & Svirbely, 1977), the use of PUVA therapy in the treatment of this condition seemed logical.

The present study describes the beneficial effect of oral photochemotherapy with methoxsalen and UVA in patients with LP. Histological examination of lesional skin before and after treatment and ultrastructural examination in the course of treatment were carried out.

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MATERIAL AND METHODS

Subjects

Seven patients (5 females and 2 males) with widespread lesions of LP were studied. In each case the diagnosis was confirmed histologically. Clinical data concerning these patients are summarized in Table 1. None of the patients received any treatment, topical or systemic, in the month prior to PUVA therapy.

TABLE 1. Data for patients undergoing photochemotherapy and treatment schedule

Patient	Age/sex	Skin type	Duration	Nc. of sessions	Frequency of PUVA exposures		Total UVA doses (J/cm ²)
1	20/F	II	6 M	27	3/WK	4/WK	58
2	50/F	III	4 M	32	3/WK		168
3	8/M	III	3 M	28	3/WK		230
4	69/F	IV	4 M	21	3/WK		107
5	8/M	II	1 M	30	3/WK	5/WK	155
6	49/F	III	2 Y	30	3/WK	5/WK	163
7	35/F	III	1 M	30	3/WK	5/WK	126

M, month; Y, year.

Light sources

Hexagonal 'stand-up' light boxes containing 84 fluorescent bulbs mounted vertically side by side were used as UVA sources. These bulbs have a continuous spectrum of high intensity irradiation between 320 and 400 nm with a peak emission at 365 nm. The energy delivered was on average 4-4.5 mW/cm² at a distance of 50 cm from the bulbs. Dosimetric control of emitted energy was regularly carried out.

Exposures and dosages (Table 1).

Methoxsalen. Ten mg capsules of methoxsalen were given 2h prior to UVA exposure according to the patient's weight: 25 kg or less, 10 mg of 8 MOP; 26-45 kg 20 mg of 8 MOP; 46-60 kg, 30 mg of 8 MOP; 60-75 kg, 40 mg of 8 MOP; 76-90 kg, 50 mg of 8 MOP; greater than 90 kg: 60 mg of 8 MOP.

UVA dosage. Initial exposure times were based on the skin type (Fitzpatrick, 1975). The initial UVA dose was 1.5-2 J/cm² and was increased at the first and third sessions of each week. The maximum dose administered at the end of treatment never exceeded 7 J/cm² in a single session. The patients were treated three times per week with an interval of 48 h between each session. Where the patient's tolerance to UVA permitted, the number of weekly sessions was progressively increased to 4 and subsequently 5. After the initial programme of treatment, maintenance therapy was not pursued.

EVALUATION OF RESULTS

Clinical. Clinical response was evaluated weekly. Two parameters were monitored: the degree of pruritus and the palpability of the lesions. The results were recorded as follows: excellent (complete clearing with total flattening of the papules accompanied by residual pigmentation and disappearance of pruritus), good (partial clearing based on diminution of clinical infiltration of lesions), fair (slight

improvement) and poor (no improvement). The patients' comments and symptoms were also recorded. Photographs were taken of each patient prior to therapy and at weekly intervals.

Histology: Biopsies were taken from all patients before treatment. In one of the 7 cases, the histology was that of lichen planopilaris.

In 6 of the 7 patients, the skin was biopsied at the end of treatment. The results of histological grading were recorded as follows: excellent (disappearance of epidermal abnormalities, and dermal infiltrate), good (disappearance of epidermal abnormalities, particularly hypergranulosis; persistence of mild non-epidermotropic infiltrate), fair (persistence of focal hypergranulosis and epidermotropism with a moderate dermal lymphocyte infiltrate), poor (no improvement).

In addition to routine light microscopy, the following techniques were undertaken on biopsy material at the end of treatment.

Immunofluorescence. In three patients, direct immunofluorescence studies were performed, before and after photochemotherapy, using standard techniques with fluorescent conjugates (Institut Pasteur), i.e. anti IgA, anti IgM, anti IgG on frozen sections.

Electron microscopy. In one case (Case 1) a biopsy of an abdominal lesion was subjected to ultrastructural examination, after a total dose of UVA of 43 J/cm^2 . The biopsy specimens were fixed in 3% glutaraldehyde for 1 h then postfixed in osmic acid for 1 h, dehydrated in alcohol, embedded in Epon and sectioned on a Reichert ultramicrotome. The specimens were stained with uranyl acetate and examined with a Hitachi HU 12 electron microscope.

RESULTS

Clinical (Fig. 1)

In 6 of the 7 patients treated, the clinical results were considered to be excellent or good (Table 2). After about 2 weeks of treatment, the lesions began to subside. During resolution they developed the appearance of fine powdery hyperkeratosis. At the end of treatment, the lesions appeared only as hyperpigmented macules with variable superficial atrophy. In the seventh patient (Case 4) the response was only fair. However, the treatment was stopped after a total dose of 107 J/cm^2 which is lower than was received by the other patients. The treatment of this patient of skin type IV would have required much greater doses of UVA. Localized lesions on the elbows or on the shins react much more slowly than others. No deterioration was seen in any patient.

Pruritus disappeared between the second and the fifth week of treatment. The mean dose of UVA required for clearing, calculated from the cases with a good response, was $150 \pm 39 \text{ J/cm}^2$ corresponding to 31 sessions of therapy. With three sessions per week, the average duration of treatment was 10 weeks.

No relapses have occurred 2–8 months after stopping treatment. Approximately 4–6 weeks after the end of therapy, the mild cutaneous atrophy was no longer detectable. The mucosal lesions persist unchanged.

Undesirable side effects of treatment were nausea in 5 patients and localized blistering in one patient. Marked dryness of the skin was observed in all the patients.

Histological findings (Table 3) (Fig. 2)

At the end of treatment, in all cases, the epidermis showed fairly marked orthohyperkeratosis. The granular layer was preserved but not hypertrophic. The Malpighian layer was generally atrophic but of

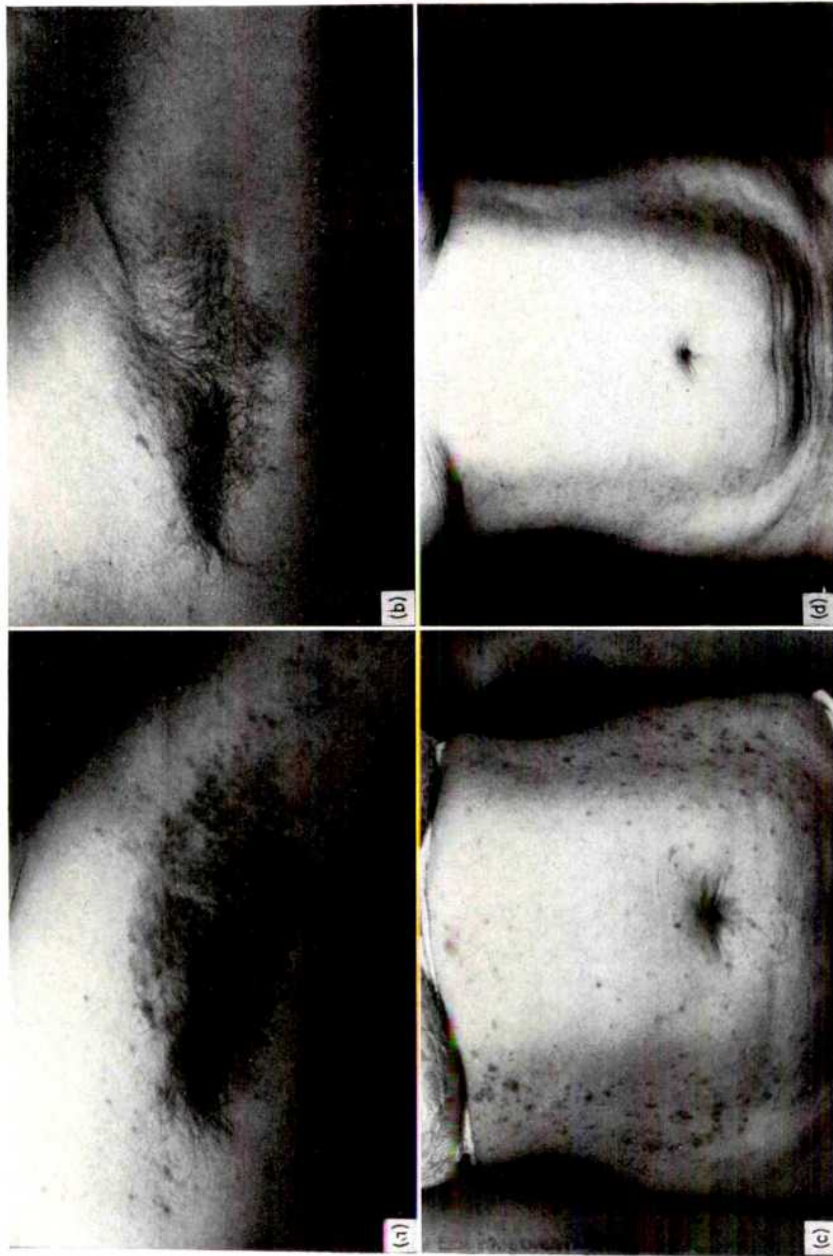


FIGURE 1. Clinical appearance before (a,c) and after (b,d) PUVA therapy.

TABLE 2. Overall results

Patient	Clinical result	Histological result	Pruritus
I	Complete clearing excellent	Good	(6)
II	Complete clearing excellent	Excellent	(16)
III	Complete clearing excellent	N.D.	(?)
IV	Fair	Fair	—
V	Complete clearing excellent	Good	(8)
VI	Complete clearing excellent	Good	(15)
VII	Complete clearing excellent	Good	(3)

() Number of sessions required for the improvement of pruritus.

normal architecture. The basal layer, however showed marked vacuolar change and the dermo-epidermal junction frequently exhibited loss of rete ridge pattern. The basement membrane was thickened in many cases and in places appeared folded. In some cases, hyaline bodies were observed. Prominent melanin pigmentation was irregularly distributed through the lower half of the epidermis. In addition, there was considerable pigmentary incontinence in the form of large deposits of intra and extracellular melanin. The papillary and upper reticular dermis were either oedematous or hyalinized. In the majority of cases, very deep alterations of elastic tissue were observed. In the papillary dermis the elastica was very fragmented.

In the reticular dermis the fibres were thinned and sometimes fragmented. In one case, atrophic hair follicles were replaced by fibrous tissue. The walls of superficial dermal capillaries were generally thickened and occasionally folded, and they were frequently surrounded by a mild mononuclear infiltrate. The deep dermis did not show any obvious pathological changes.

Immunofluorescence

In the three cases studied, fluorescence of Civatte bodies was seen particularly with an anti IgM conjugate and very weakly with anti IgG and anti IgA conjugates.

At the end of treatment this fluorescence had disappeared in one case and persisted unchanged in the other two.

Electron microscopy

Epidermis. In the granular layer abnormal keratinocytes with a deeply indented nucleus were observed. In certain sections, these nuclei appeared to consist of two, three or four separate lobules. The nucleoli, at times multiple (2 or 3), were always prominent. Heterochromatin was scarce or even absent. Euchromatin at times occupied the entire nucleus. The nuclear membrane was intact. On the other hand, the plasma membrane had disappeared. Keratohyalin granules were quantitatively and qualitatively normal.

In the Malpighian layer there was widening of intercellular spaces without rupture of desmosome-tonofilament complexes. The nuclei showed prominent indentations with always one or two nucleoli. Occasionally the cytoplasm appeared vacuolated.



FIGURE 2. Histology before (a,c) and after (b,d) PUVA therapy.

TABLE 3. Histological observations after PUVA therapy

Patients	Hyperkeratosis	Epidermis		Dermal infiltrate
		Vacuolization of basal layer cells	Epidermal atrophy	Density %
I	++	++	+ or absent	(90)
II	+	NONE	+ or absent	(100)
III	N.D.	N.D.	N.D.	N.D.
IV	+++	+++	+ or absent	(60)
V	++	+	+ or absent	(90)
VI	+	+++	+ or absent	(90)
VII	++	+	+ or absent	(90)

N.D.: not done.

(%): % of the decrease in the dermal infiltrate.

Cellular alteration was particularly obvious in the basal layer (Fig. 3). In some keratinocytes, tonofilaments formed thick perinuclear bundles. Occasional keratinocytes were totally necrosed and numerous masses of filaments, not limited by a membrane and sometimes containing mature melanosomes, could be observed. The melanocytes were generally very active and contained mostly mature melanosomes situated almost entirely in the peripheral cytoplasm. Likewise the numerous dendrites were packed with mature melanosomes. The basement membrane was frequently ruptured. In other places it was convoluted or folded.

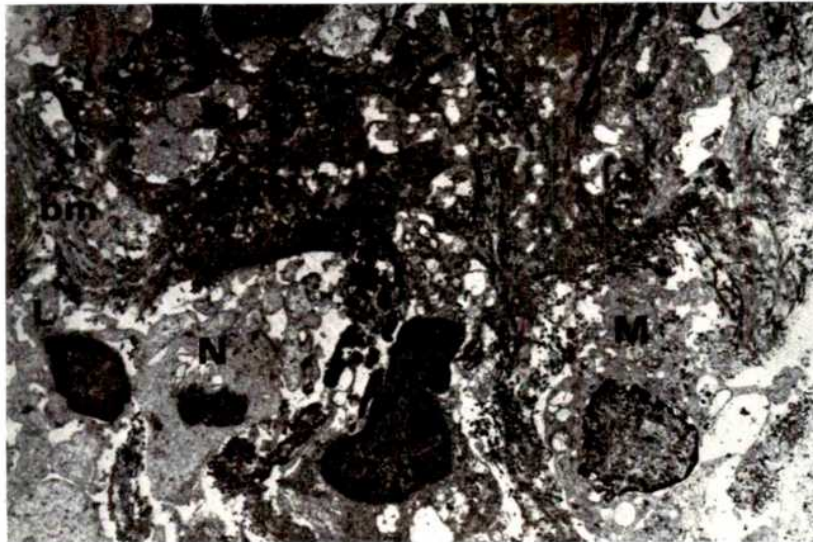


FIGURE 3. Severe cellular changes in the basal layer with vacuolated cytoplasm of melanocytes. (L: altered lymphocyte; n: necrotic cell) ($\times 5400$).

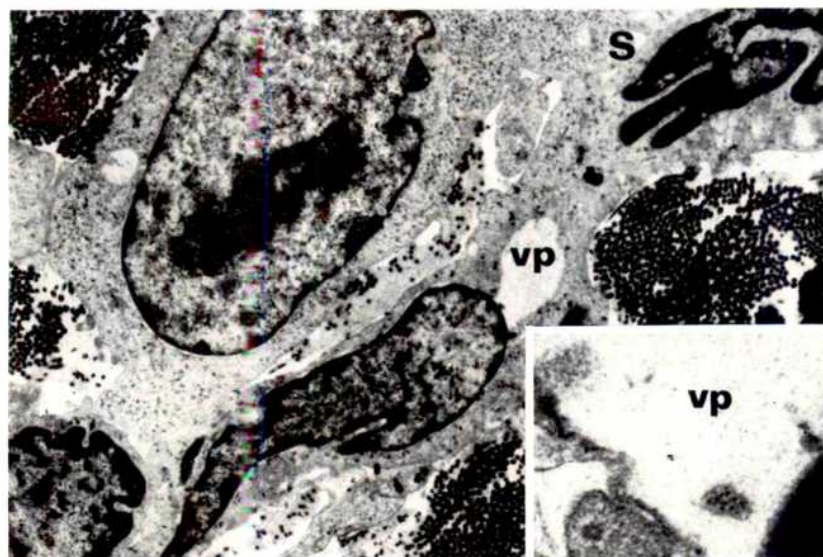


FIGURE 4. Dilatation of the perinuclear space in mid-dermal mononuclear cell (S: Sézary like cell) ($\times 9000$). Inset: high-power view of the perinuclear vacuole ($\times 54000$).

Dermis. The superficial dermis also revealed marked pathological changes. Collagen bundles were separated by considerable oedema. The nuclei of fibroblasts and macrophages were indented and showed multiple convolutions (Figs 5–8). In places the perinuclear space was dilated giving a vacuolar appearance. Very large intracytoplasmic vacuoles were frequently observed. These were bounded by a discontinuous membrane and tended to squash the nucleus. Mitochondria were generally dilated.

A few cells resembling Sézary cells were also present in the superficial dermis (Fig. 4). Some of them showed dilatation of the perinuclear space. Others did not reveal nuclear or cytoplasmic abnormalities.

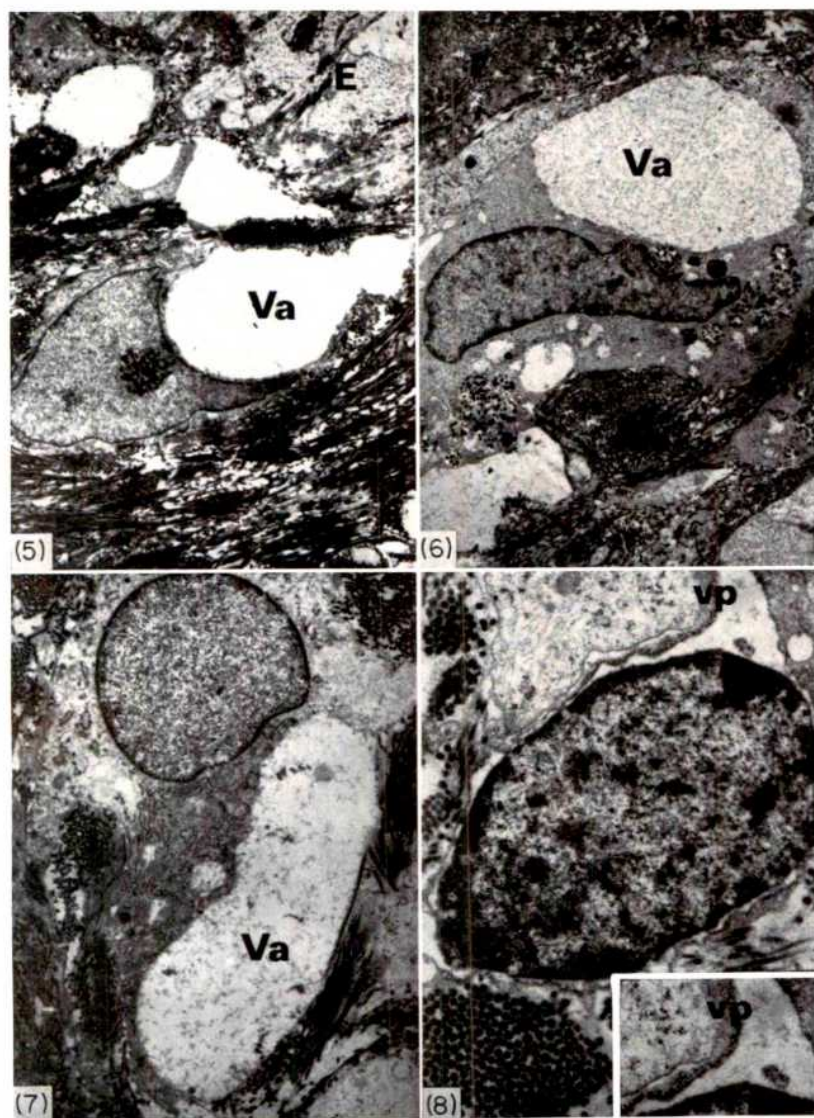
The deep reticular dermis was normal.

DISCUSSION

Lichen planus frequently presents a difficult therapeutic problem. The natural history of the condition without treatment is usually prolonged and spontaneous resolution is not invariable (Altmann & Perry, 1961).

Oral photochemotherapy seems to be an efficient mode of treatment, improving both the itching and the clinical lesions which become progressively less palpable and finally imperceptible at the end of treatment. Our results agree with Hönigsman *et al.* (1977) who stated that photochemotherapy was effective in lichen planus. Histology confirms the disappearance of the superficial dermal infiltrate. This regression of lichen planus seen in 6 out of 7 patients is unquestionably due to photochemotherapy. Indeed, at the end of therapy, a few lichen planus papules may persist in non-exposed areas (inner aspect of the thighs) in many patients. This fact and the persistence unchanged of mucosal lesions eliminates the possibility of spontaneous resolution in these cases.

The doses required for clearing are about double those required for clearing psoriasis. In our experience they are comparable to those required for the effective treatment of mycosis fungoides. The responses obtained seem to be maintained without further treatment.



FIGURES 5-8. Cellular changes in the upper dermis. Fig. 5. Large intercytoplasmic vacuoles (Va) compressing the nucleus of a fibroblast (E: epidermis) ($\times 6000$). Fig. 6. Intracytoplasmic vacuole (Va) in a melanophage (Mp: melanophagolysosomes) ($\times 4800$). Fig. 7. Intracytoplasmic vacuole (Va) in a fibroblast. The cell membrane is destroyed ($\times 9600$). Fig. 8. Dilation (Vp) of the perinuclear space of a mononuclear cell of the dermal infiltrate ($\times 20,000$). Inset: high power view of the perinuclear vacuole ($\times 36,000$).

Histological examination carried out after treatment shows profound alterations in the superficial dermis, particularly of the elastic fibres. These changes which are not usually present in lichen planus appear to be due to photochemotherapy. The changes in elastic tissue have not been seen in psoriatics treated with PUVA (Braun Falco, Hofmann & Plewig, 1977). It must be emphasized that our patients received a total dose of UVA considerably in excess of that normally used in the treatment of psoriasis.

Besides the disappearance of the dermal infiltrate, photochemotherapy produces marked changes in the epidermis of the lesion. The thickness of the granular layer diminishes considerably, as does the thickness of the Malpighian layer. However, photochemotherapy is probably not the sole cause of epidermal atrophy seen in lichen planus lesions after treatment. Normal epidermis usually reacts to photochemotherapy by hyperplasia and 'pseudoparakeratosis' (Toda, Nakayama & Morikawa, 1974). This tendency to atrophy after PUVA appears to be specific to lichen planus, further supporting the theory of an epidermal origin for lichen planus.

We did not find keratohyalin granules in the keratinocytes of the lower half of the Malpighian layer, such as were described in normal epidermis after topical trimethylpsoralen (TMP) and UVA (Toda *et al.*, 1974).

Ultrastructural study shows changes of the epidermis and superficial dermis which can be classified in two groups:

(1) Anomalies which are known to be due to lichen planus and for which photochemotherapy is not responsible: colloid bodies, intercellular oedema, changes in desmosome-tonofilament complexes, rupture of the basement membrane (Medecina & Lorincz, 1977).

(2) Anomalies which may be due to photochemotherapy. The nuclear alterations seen in some keratinocytes (nuclear lobulation, modification of chromatin distribution) probably belong to this group as they have not been observed in untreated lichen planus (Medecina & Lorincz, 1977). Nucleolar and chromatin changes are observed transitorily in guinea-pig epidermis after injection of 8 MOP and UVA irradiation (Mizuno *et al.*, 1974). The indented appearance of keratinocyte nuclei in the deep part of the Malpighian layer was seen in human epidermis a few hours after local application of TMP followed by UVA irradiation (Toda *et al.*, 1974). Specimens which were taken from uninvolved skin of psoriatic patients who had been treated between 20 and 40 times with an oral dose of 8 MOP and UVA irradiation revealed the presence of binucleate, tetranucleate and multinucleate epidermal cells (Omar, Wiesmann & Krebs, 1977). It is therefore probable that keratinocyte nuclei are one of the targets of PUVA therapy.

The nuclear or cytoplasmic changes seen in cells of various type (melanophages, fibroblasts, lymphocytes, Sézariiform cells) which populate the superficial dermis in lichen planus are probably due to PUVA therapy. The cells of the dermal infiltrate of untreated lichen planus do not usually show ultrastructural changes (Medecina & Lorincz, 1977) of the type we have observed. Furthermore, the changes seen in the dermal cells are limited to the superficial dermis, localized to an area corresponding to that which is usually considered to be the limit of penetration of UVA in human skin (Everett *et al.*, 1966). Nuclear anomalies of superficial dermal fibroblasts have been observed previously in patients treated by PUVA therapy (Omar *et al.*, 1977). The cytoplasmic vacuolization which we have found only in cells of the basal layer and superficial dermis may be compared to that which has been described in psoriatic epidermis treated by photochemotherapy (Vukas, Velfi, & Gligora, 1977). It doubtless constitutes a sign of cellular damage caused by photochemotherapy.

The aetiology of lichen planus is unknown. It is therefore difficult to conjecture on the mode of action of photochemotherapy in this condition. An autoimmune mechanism has been suggested. It has been postulated that in lichen planus, degenerating epidermal cells stimulate a brisk inflammatory response with resultant hydrolysis of the colloid bodies (Black, 1971, 1972). Lymphocyte transformation has been demonstrated in lymphocytes cultivated in the presence of tissue extracts of lichen planus (Sarkany & Gaylarde, 1971). Several ultrastructural studies (Johnson & Fry, 1967; Sarkany & Gaylarde, 1971; Ebner, 1973; Medecina & Lorincz, 1977) seem to show that in the evolution of lichen planus epidermal changes precede dermal changes, particularly the appearance of the infiltrate. It has therefore been suggested that the initial change in lichen planus is situated in the keratinocyte nucleus of the germinative layer and happens during the initial phase of mitosis. Then an antigen situated at

this level may be the origin of a cellular immune response. According to this hypothesis two principal factors are involved: the appearance of a new epidermal antigen and the appearance of a dermal lymphocytic infiltrate. Our ultrastructural findings suggest that photochemotherapy may act on both of these factors. It is possible that the nuclear changes induced in the keratinocytes may modify the appearance of the hypothetical epidermal antigens responsible for the development of lichen planus. Likewise the nuclear and cytoplasmic changes found in the superficial dermal infiltrate suggest that photochemotherapy may have a direct ablative action on the lymphocytes. These hypotheses which rely on morphological data can only be considered as a basis for further study.

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REFERENCES

- ALARIO, A., ORTONNE, J.P., SCHMITT, D. & THIVOLET, J. (1978) Lichen planus (LP): study with anti human T lymphocyte antigen (anti HTLA) serum on frozen tissue sections. *British Journal of Dermatology*, **98**, 601.
- ALTMAN, J. & PERRY, H.O. (1961) Variations and course of lichen planus. *Archives of Dermatology*, **84**, 179.
- BLACK, M.M. (1971) The role of the epidermis in the histopathogenesis of lichen amyloidosis. Histochemical correlations. *British Journal of Dermatology*, **85**, 254.
- BLACK, M.M. (1972) The pathogenesis of lichen planus. *British Journal of Dermatology*, **86**, 302.
- BRAUN FALCO, O., HOFMANN, C. & LEWIG, G. (1977) Feingewebliche Veränderungen unter Photochemotherapie der Psoriasis: eine histologische und histochemische Studie. *Archives for Dermatological Research*, **257**, 307.
- EBNER, H. (1973) Untersuchungen über die cellulare Zusammensetzung des Lichen ruber planus Infiltrate. *Archives for Dermatological Research*, **247**, 309.
- EVERETT, M.A., YEARGERS, E., SAVRE, R.M. & OLSON, R.L. (1966) Penetration of epidermis by ultraviolet rays. *Photochemistry and Photobiology*, **5**, 533.
- FITZPATRICK, T.B. (1975) *Medical Problems of Enhanced Ultraviolet Radiation in Stratospheric Ozone Depletion*. Hearing before the subcommittee in the upper atmosphere of the Committee on aeronautical and space sciences. United States Senate, 99th Congress printed for the use of the Committee on Aeronautical and Space Sciences U.S. Government Printing Office Washington, 464.
- GILCHRIST, B.A., PARRISH, J.A., TANENBAUM, L., HAYNES, H.A. & FITZPATRICK, T.B. (1976) Oral methoxsalen photochemotherapy of mycosis fungoides. *Cancer*, **38**, 683.
- HJORTSHØJ, A. & SCHMIDT, H. (1977) Photochemotherapy of mycosis fungoides. *Danish Medical Bulletin*, **24**, 40.
- HONIGSMANN, H., GSCHNITZ, F., KONRAD, K. & WOLFF, K. (1977) Photochemotherapy for pustular psoriasis (Von Zumbusch). *British Journal of Dermatology*, **97**, 119.
- JOHNSON, F.R. & FRY, L. (1967) Ultrastructural observations in lichen planus. *Archives of Dermatology*, **95**, 596.
- MEDECINA, M. & LORINCZ, A. (1977) Lichen planus: an ultrastructural study. *Acta dermato-venereologica*, **57**, 55.
- MIZUNO, N., TSUNEISHI, S., MATSUHASHI, S., KIMURA, S., FUJIMURA, Y. & USHIJIMA, T. (1974) Some aspects on the action mechanism of 8-methoxypsoralen photosensibilization. In: *Sunlight and Man* (Ed. by M.A. Pathak *et al.*) p. 389. University of Tokyo Press.
- OMAR, A., WISMANN, U.N. & KREBS, A. (1977) Induction of multinucleate cells by 8 MOP and UV treatment *in vitro* and *in vivo*. *Dermatologica*, **155**, 65.
- ROENIGK, H.H., JR. (1977) Photochemotherapy for mycosis fungoides. *Archives of Dermatology*, **113**, 1047.
- SARKANY, I. & GAYLARDE, P.M. (1971) Ultrastructural and light microscopic changes of the epidermal junction. *Transactions of the St Johns Hospital Dermatological Society*, **57**, 139.
- SHOUKHA, S. & SVIRHELY, Y. (1977) Immunohistochemical study of lichen planus. *Journal of Clinical Pathology*, **30**, 569.
- TAN, R.S., BYROM, N.A. & HAYES, J.P. (1975) A method of liberating living cells from the dermal infiltrate. Studies on skin reticuloses and lichen planus. *British Journal of Dermatology*, **93**, 271.
- TODA, K., NAKAYAMA, Y. & MORIKAWA, F. (1974) Electron microscopic. Observations in human skin after Psoralen photosensibilization. In: *Sunlight and Man* (Ed. by M.A. Pathak *et al.*), p. 419. University of Tokyo Press.

Suppression of epidermal proliferation by ultraviolet light, coal tar and anthralin

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SUMMARY

360 nm ultraviolet light (UVA) plus coal tar depresses mitosis and DNA synthesis in epidermal cells. UVA has no effect by itself. In some models coal tar alone can partially depress DNA synthesis. 254 nm ultraviolet light (UVC) and 290–320 nm ultraviolet light (UVB) do not enhance depression of DNA synthesis by coal tar. Dithranol will suppress mitosis and DNA synthesis of epidermal cells but there appears to be no photo-activation by UVA. Dithranol suppression and suppression of DNA synthesis by UVB or UVC are additive when used together.

Although it has been over 50 years since Goeckerman (1925) introduced crude coal tar and ultraviolet light (UVL) for psoriasis, the cellular or molecular mechanisms of action of coal tar in skin diseases are only dimly perceived. Although tar therapy is ancient it is still actively used in modern practice, particularly in eczematous dermatitis and psoriasis. There are probably hundreds of different chemical entities in coal tar but the specific chemicals which are active against skin disease are unknown.

It is the purpose of this paper to report the following: (i) The ability of crude coal tar and a refined coal tar product, Estar gel, to inhibit cellular deoxyribonucleic acid (DNA) synthesis and mitosis *in vivo* especially when combined with long wave ultraviolet light (UVA). (ii) The role of dithranol in inhibiting DNA synthesis and mitosis of epidermal cells of normal mice with and without the addition of 254 nm light (UVC), 290–320 nm light (UVB), or 360 nm light (UVA). (iii) The inhibition of mitosis and DNA synthesis in models of stimulated epidermal proliferation. This includes hairless mice with essential fatty acid deficiency and hairless mice with previous UVC exposure. The effect on DNA synthesis and mitotic index of coal tar and dithranol, with and without ultraviolet light, is examined.

Recent reports indicate a correlation of the ability of topical drugs to inhibit DNA synthesis and mitosis in hairless mouse epidermis with their ability to clear plaques of psoriasis (du Vivier *et al.*, 1976). We believe this report demonstrates depression of DNA synthesis and mitosis by coal tar with UVA and dithranol without UVA and this correlates with their ability to suppress psoriatic lesions and perhaps those of eczematous disorders.

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METHODS

Hairless mice HRS/J were used for all experiments. These mice have been propagated from our own breeding colonies. Most experiments were conducted with animals between 1 and 4 months of age. Randomization of animals was carried out in all experiments.

Light sources

UVA. A Blak-Ray B-100A lamp (Ultra-Violet Products, Inc., San Gabriel, California 91778) with maximum output in the 360 nm range was used throughout and calibrated regularly with a Blak-Ray UVA meter (Model J-221). The animals were exposed at a distance of 25 cm from the lamp which gave a radiation dose of 6.7 J/cm² over a 45 min exposure.

UVB. Two FS-40 Westinghouse lamps were used at a distance of 30 cm. The minimal erythema dose for skin of medium pigmentation of Caucasians was 5–10 min at 30 cm. A dose response was established for depression of DNA synthesis *in vivo* in mouse skin (Table 1). These lamps emit from 275 to 380 nm but with a broad peak at 313 nm.

UVC. A General Electric germicidal lamp (G8T5) with output of 12 μ W/cm² was used at a distance of 15 cm. The primary output is at 254 nm. A dose response was established for depression of DNA synthesis *in vivo* in mouse skin (Table 1).

In vivo models of stimulated mouse epidermis

(a) Essential fatty acid deficient (EFAD) mice were developed as stated in a previous publication (Lowe, 1977). The deficiency was fully established in mice used for these experiments after approxi-

TABLE 1. DNA synthesis of normal mouse skin with different exposure levels of UVA, UVB and UVC without tar preparations*

	Time of exposure (min)	dpm/ μ g DNA $\pm$ s.d.
UVA (target-skin distance is 25 cm)	45	67.6 $\pm$ 13.4
	0	72.0 $\pm$ 4.7
UVB† (target-skin distance is 30 cm)	27	6.9 $\pm$ 3.1‡
	9	21.5 $\pm$ 9.7‡
	3	59.5 $\pm$ 9.7
	1	68.8 $\pm$ 21.2
	0	58.6 $\pm$ 5.3
UVC (target-skin distance is 15 cm)	27	13.4 $\pm$ 5.9‡
	9	28.7 $\pm$ 5.0‡
	3	29.9 $\pm$ 4.0‡
	1	55.8 $\pm$ 8.1‡
	0	95.6 $\pm$ 14.0

* Killed 4 h after irradiation—5 animals per group.

† Minimal erythema dose for medium pigmented Caucasian skin is 5–10 min at 30 cm.

‡ Significant depression $P < 0.05$.

Threshold dose for suppression of DNA synthesis by UVB is in range of 3–9 min and that for UVC is about 1 min at distances related. Exposures to UVA up to 45 min do not depress DNA synthesis.

mately 60 days of diet, when incorporation of tritiated thymidine and mitotic index was more than double that of matched normal diet mice.

(b) Epidermis stimulated *in vivo* by UVC was used for some of the experiments. The techniques used for UVC exposure and recording the initial inhibition, with the subsequent stimulation at 48 h, of mitotic index and DNA synthesis have been reported previously (du Vivier *et al.*, 1976).

Coal tar preparations

5% crude coal tar in petrolatum was obtained from our pharmacy. Estar gel (Westwood Pharmaceuticals Inc., Buffalo, New York 14213) is a commercial 0.6% coal tar preparation which has been partially refined and put into an alcoholic propylene glycol gel vehicle. Controls were skin to which the vehicles for these tar preparations were applied or skin which had nothing applied to it.

Dithranol

Dithranol 0.4% (Lasan Unguent, Stiefel Laboratories, Inc., Oak Hill, New York 12460) was applied in the same way as the tar preparations.

Sequence of events

For most experiments, a thin layer of the preparations was applied to the back with a cotton applicator and the animal immobilized for 2 h. Then the area was washed with Purpose soap, rinsed with distilled water and gently patted dry. The animals were then exposed to the light source for the appropriate time. At 1 or 4 h following light exposure the animals were injected intraperitoneally with 25 μ Ci tritiated thymidine (6 μ g thymidine) and killed 1 h after the injection.

Samples were taken at the treated site for (i) determination of specific activity of epidermal DNA and (ii) determination of mitotic index or labelling index as previously published (du Vivier *et al.*,

TABLE 2. Suppression of DNA synthesis by 5% crude coal tar and UVA applied to EFAD epidermis*

	dpm/ μ g DNA $\pm$ s.d.
1. 5% Crude coal tar	57.3 $\pm$ 12.8
2. 5% Crude coal tar + UVA (45 min)	22.7 $\pm$ 6.2
3. Vehicle + UVA (45 min)	113.1 $\pm$ 18.1
4. UVA only (45 min)	116.2 $\pm$ 21.8
5. Vehicle only	121.8 $\pm$ 19.6

* Mice maintained on essential fatty acid deficient (EFAD) diet to stimulate epidermal proliferation. Five hairless mice for each group—killed 4 h after irradiation. Significant depression of DNA synthesis was observed with 5% crude coal tar + UVA (45 min) ($P < 0.01$) and with 5% crude coal tar alone ($P < 0.05$). There is a significant difference between 5% crude coal tar alone and 5% crude coal tar + UVA ($P < 0.05$).

1976). Specific activity was expressed as disintegrations per minute (dpm) per μg of DNA as determined using a Beckman LS-150 liquid scintillation counter with an efficiency for tritium of 32.1%.

Statistical analysis

Student's 't'-test for unpaired data with a two-sided hypothesis was used.

RESULTS

Table 1 outlines the dose response for suppression of DNA synthesis by UVA, UVB and UVC without any tar preparations applied to the skin. UVA did not result in any suppression of DNA synthesis in the absence of tar preparations. At a skin-target distance of 30 cm UVB gave marked suppression of DNA synthesis between 3 and 9 min of exposure. This is the range of one erythema dose in medium pigmented Caucasians. The skin-target distance of 15 cm with the UVC source gave significant depression of DNA synthesis after 1 min of exposure.

Crude coal tar alone and crude coal tar plus UVA significantly depress DNA synthesis in the EFAD model (Table 2). The inhibition is greater with crude coal tar plus UVA than it is with crude coal tar alone. This suggests that there is a factor in tar which depresses DNA synthesis without the

TABLE 3. Effect of dithranol 0.4% with and without ultraviolet light on DNA synthesis and mitosis

	dpm/ μg DNA $\pm$ s.d.	Mitotic index $\pm$ s.d.
1. Dithranol 0.4% only	25.2 $\pm$ 12.9	7 $\pm$ 2
2. Dithranol 0.4% + UVA (45 min)	25.6 $\pm$ 9.7	5 $\pm$ 5
3. Dithranol 0.4% + UVB (4 min)	7.5 $\pm$ 1.6	ND
4. Dithranol 0.4% + UVC (45 s)	10.0 $\pm$ 1.6	ND
5. UVA only (45 min)	67.6 $\pm$ 13.4	30 $\pm$ 12
6. UVB only (4 min)	36.8 $\pm$ 5.6	ND
7. UVC only (45 s)	50.8 $\pm$ 9.3	ND
8. Control (vehicle only) (no UV light)	72.0 $\pm$ 4.7	31 $\pm$ 16

Low doses of either UVB or UVC alone give a slight but significant depression of DNA synthesis. Dithranol plus UVB or UVC gives greater suppression than with dithranol alone suggesting a simple additive effect as dithranol alone also depresses DNA synthesis. UVA in doses which clearly photoactivate crude coal tar to inhibit DNA synthesis do not enhance effect of dithranol on DNA synthesis. 1 vs 2—no significant difference. 1 and 2 vs 3—significant $P < 0.01$. 1 vs 3 and 4—significant $P < 0.05$. 3 vs 6—significant $P < 0.01$. 4 vs 7—significant $P < 0.01$. 1 and 2 vs 5—significant $P < 0.01$. Five animals per group—killed 4 h after irradiation.

Normal hairless mice were used.

ND = Not done.

TABLE 4. Suppression of DNA synthesis and mitosis in UVC stimulated mouse epidermis by Estar gel and UVA*

	dpm/ μ g DNA $\pm$ s.d.	Labelling index $\pm$ s.d.	Mitotic index $\pm$ s.d.
Killed 1 h after UVA			
1. Estar gel + UVA†	35.5 $\pm$ 20.9	ND	3 $\pm$ 3
2. Estar gel only	188.7 $\pm$ 57.0	ND	69 $\pm$ 26
3. UVA only	196.6 $\pm$ 10.3	ND	48 $\pm$ 22
4. Control (gel base only)	194.4 $\pm$ 76.6	ND	85 $\pm$ 24
Killed 4 h after UVA			
1. Estar gel + UVA‡	12.5 $\pm$ 6.9	1 $\pm$ 1	4 $\pm$ 1
2. Estar gel only	70.7 $\pm$ 42.1	163 $\pm$ 54	55 $\pm$ 14
3. UVA only	78.8 $\pm$ 28.3	202 $\pm$ 124	56 $\pm$ 21
4. Control (gel base only)	66.7 $\pm$ 15.3	135 $\pm$ 42	48 $\pm$ 20

* Mice treated 48 h previously with UVC to stimulate epidermal proliferation. Animals were killed either 1 or 4 h after UVA exposure.

† Group 1 different from 2, 3, 4 for dpm/ μ g DNA, labelling index, and mitotic index: $P < 0.01$ for all comparisons. Four animals per group.

‡ Group 1 different from 2, 3, 4 for dpm/ μ g DNA, labelling index, and mitotic index: $P < 0.001$ for all comparisons. Ten animals per group.

ND = not done.

benefit of UVA and a factor which acts in conjunction with UVA to depress DNA synthesis further.

Table 3 explores the effect of dithranol 0.4% with and without UVB, UVC or UVA. It is evident that dithranol alone gives a significant depression of DNA synthesis and mitotic index. When UVA is added to dithranol treated skin, there is no increase in the depression of DNA synthesis or mitotic index. When UVB or UVC is added to the dithranol treated sites there appears to be a combined effect which is additive. That is, the UVB and UVC depression of DNA synthesis seem to be added to the effect of dithranol alone. There is no evidence that UVC, UVB or UVA photoactivate dithranol.

Estar gel when combined with UVA exposure also causes a marked suppression of DNA synthesis in the UVC stimulated model (Table 4). However, in this UVC stimulated model there is no evidence for suppression of DNA synthesis by Estar gel without UVA. This depression of DNA synthesis is present 1 and 4 h following exposure to UVA. Also there is suppression of the mitotic index by Estar gel plus UVA. This suppression is as great at 2 h post-irradiation as it is at 5 h post-irradiation suggesting that the effect is on G₂ as well as on the S phase (DNA synthesis) of the cell cycle. The labelling index shows the same decrease with Estar gel plus UVA as do the mitotic index and DNA synthesis as measured with the hydroxyapatite extraction technique. The specific activity of the DNA is lower in the 4 h post-irradiation group than in the 1 h post-irradiation group. However there is frequent variation in the uptake in controls which so far we have not been able to correlate with any particular factor. Thus each experiment with each group of animals has to have its simultaneous controls and statistical significance determined within these groups in each experiment.

Estar gel will significantly depress DNA synthesis in the EFAD model both when combined with UVA and without UVA (Table 5). There is a tendency for the Estar gel plus UVA to depress DNA synthesis more than Estar gel alone but it is not of statistical significance in this experiment which included only 5 animals per group. The mitotic index was significantly depressed in the Estar gel plus UVA group as compared to UVA alone. Again, there was a tendency for the mitotic index of the Estar gel only group to be lower than the controls of UVA only and placebo vehicle only but not of statistical significance in these relatively small groups. The lack of effect of Estar gel alone in UVC

TABLE 5. Suppression of DNA synthesis and mitosis in EFAD mouse epidermis by Estar gel and UVA*

	dpm/ μ g DNA $\pm$ s.d.	Mitotic index $\pm$ s.d.
1. Estar gel + UVA	6.5 $\pm$ 8.4	26 $\pm$ 19
2. Estar gel only	29.9 $\pm$ 9.3	39 $\pm$ 25
3. UVA only	39.4 $\pm$ 35.5	74 $\pm$ 13
4. Control (gel base only)	36.6 $\pm$ 11.8	67 $\pm$ 30

* Mice maintained on essential fatty acid deficient (EFAD) diet to stimulate epidermal proliferation. Five animals per group—killed 4 h after UVA exposure.

Mitotic index—1 vs 3 and 2 vs 3 $P < 0.01$. No significant difference between the others.

dpm/ μ g DNA—1 vs 3 and 4 $P < 0.01$. 2 vs 3 and 4 $P < 0.01$.

TABLE 6. Dose response showing relation of suppression of DNA synthesis to amount of UVA exposure of skin treated with Estar gel. Normal hairless mouse skin was used. Exposure of 6.7 min gave one J/cm²

	Time of exposure (min)	dpm/ μ g DNA $\pm$ s.d.
UVA + Estar gel		
(target-skin distance is 30 cm)	45*	24.9 $\pm$ 14.3
	20*	28.7 $\pm$ 14.3
	10*	35.2 $\pm$ 11.8
	5	60.4 $\pm$ 23.4
	2	115.9 $\pm$ 35.2
	0	101.9 $\pm$ 30.5

* Statistically significant depression $P < 0.01$ as compared to 0 min (control). Five animals per group—killed 4 h after irradiation.

stimulated skin as compared to the depression of DNA synthesis in EFAD animals may be due to a difference in penetration of skin by agents in tar. Recent work shows an increased penetration of EFAD hairless mouse skin as compared to control hairless mice (Solomon & Lowe, submitted for publication).

A dose response of DNA suppression by Estar gel plus UVA is outlined in Table 6. One J/cm² is delivered in 6.7 min. Significant depression of DNA synthesis occurs between 5 and 10 min or in the neighbourhood of 1 J/cm².

Table 7 shows that Estar gel is not enhanced in its ability to suppress DNA synthesis when combined with UVB or UVC. Thus only UVA potentiates the activity of Estar gel on DNA synthesis. We used the smallest amount of UVB and UVC that would give some depression of DNA synthesis with these UV exposures alone. We were unable to show any enhancement of suppression of DNA synthesis by adding Estar gel before the UVB or UVC exposure.

In interpreting the rate of incorporation of tritiated thymidine into DNA, labelling indices and

TABLE 7. Mice exposed to UVB and UVC before and after application of Estar gel*

	dpm/ μ g DNA $\pm$ s.d.
1. Vehicle only	108.7 $\pm$ 21.5
2. UVB only (4 min)	55.8 $\pm$ 8.1
3. UVC only (45 s)	79.1 $\pm$ 18.4
4. Estar gel only	75.7 $\pm$ 15.0
5. Estar gel before UVB (4 min)	58.3 $\pm$ 12.5
6. Estar gel after UVB (4 min)	59.5 $\pm$ 19.0
7. Estar gel before UVC (45 s)	55.1 $\pm$ 8.4
8. Estar gel after UVC (45 s)	61.7 $\pm$ 13.4

* Five animals per group—normal mouse skin. 5 and 7—Estar gel applied 2 h before exposure to UVB or UVC. 6 and 8—UVB or UVC exposure followed by Estar gel application for 2 h. ^{3} H-thymidine injected 6 h after start of each experiment and animals killed 7 h after start of each experiment. 1 different from 2–8 $P < 0.05$. No other significant differences between groups.

mitotic indices, we may not be measuring rates of entry into DNA synthesis and mitosis but the results could be explained by changes in t_s and t_m without changes in proliferative rate.

DISCUSSION

Crude coal tar and ultraviolet light have been used in the treatment of psoriasis for the past 50 years, since introduced by Goeckerman (1925). The mechanism of action of tar and UVL has never been clearly defined but in general has been felt to be due to a phototoxic reaction (Obermayer & Becker, 1935). A similar type of phototoxic reaction with pigmentation is seen with psoralen and UVA (320–400 nm) treatment of vitiligo (Lerner, Denton & Fitzpatrick, 1953). Recently this combination has been shown to inhibit epidermal DNA synthesis (Epstein & Fukuyama, 1970; Walter *et al.*, 1973), and this may be the mechanism of action in clearing the more rapidly proliferating epidermis of psoriasis, as shown in clinical studies (Walter & Voorhees, 1973; Weber, 1974; Parrish *et al.*, 1974). Similarly, recent reports (Pathak & Biswas, 1977) would indicate that coal tar preparations in combination with UVA form DNA cross links. This correlates with the photosensitizing ability of these preparations and is similar to psoralens and UVA (Cole, 1970). Tar and psoralens with UVA may work through similar mechanisms to combine with DNA, inhibit epidermal cell proliferation and thereby improve psoriasis.

Our results show that crude coal tar and certain derivatives such as Estar gel do indeed inhibit DNA synthesis and mitosis in hairless mouse skin to a greater extent when combined with UVA. UVA alone has no effect. This would correlate clinically with the lack of response to UVA by itself in psoriasis (Fischer, 1976). Crude coal tar, dithranol and to a certain extent, Estar gel, when used alone, do cause some inhibition of epidermal proliferation. Dithranol does not appear to be photo-activated by the UVA dose used in this study. Other reports (Tanenbaum *et al.*, 1975) have shown that dithranol will elicit a phototoxic response, but probably requires higher UVA doses than we used. Dithranol by itself has previously been shown to inhibit DNA synthesis in both guinea-pig and human epidermis (Swanbeck & Liden, 1966; Liden & Michaëlsson, 1974). Both UVB (290–320 nm) and

UVC (below 290 nm) inhibit DNA synthesis and their effect with dithranol appears to be additive. UVB and UVC did not seem to enhance the DNA synthesis depression of topical Estar gel.

The results of this study suggest that the newer tar gels, in combination with a potent UVA source, might be beneficial in the treatment of psoriasis. The tar gel-UVA effect on DNA synthesis and mitosis appears to be equal to or more pronounced than crude coal tar and dithranol used alone or in combination with UVB or UVC. The tar gels however, would be less messy and more acceptable for inpatient and outpatient use.

In conclusion, the ability of tar and its derivatives to improve psoriasis may work through the inhibition of DNA synthesis, especially when photoactivated by UVA, and would indeed point to using such a mode of therapy for psoriatic patients. Whether or not this would be more efficient than using UVB or UVC light sources is not clear. Past studies showing the relative clinical ineffectiveness of tar and UVA may have been due to the low amounts of UVA used (Tanenbaum *et al.*, 1975). The hairless mouse assay system used in this study may be a valuable indicator of the potential of various tars with different types of UVL sources to inhibit epidermal proliferation. Screening with this technique could show which tar derivatives and which UVL sources alone or in combination might produce the most beneficial response. This could be a more precise tool for predicting therapeutic effectiveness than relying on clinical phototoxic reactions alone, as suggested by others (Kaidbey & Kligman, 1977). We plan to evaluate crude coal tar and its derivatives including the newer tar gels with UVA in psoriasis.

REFERENCES

- COLE, R.S. (1970) Light induced cross linking of DNA in the presence of a furocoumarin (psoralen): Studies with phage α , *Escherichia coli*, and mouse leukemia cells. *Biochimica et Biophysica Acta*, **217**, 30.
- DU VIVIER, A., BIBLE, R., JR, MIKURIYA, R.K. & STOUGHTON, R.B. (1976) An animal model for screening drugs for antipsoriatic properties using hydroxyapatite to isolate DNA rapidly from the epidermis. *British Journal of Dermatology*, **94**, 1.
- EPSTEIN, J.H. & FUKUYAMA, K. (1970) A study of 8-methoxypsoralen induced phototoxic effects on mammalian epidermal macromolecular synthesis *in vivo*. *Journal of Investigative Dermatology*, **54**, 350.
- FISCHER, T. (1976) UV-light treatment of psoriasis. *Acta dermato-venereologica*, **56**, 473.
- GOECKERMAN, W.H. (1925) Treatment of psoriasis. *Northwest Medicine*, **24**, 299.
- KAIDBEY, K.H. & KLIGMAN, A.M. (1977) Clinical and histological study of coal tar phototoxicity in humans. *Archives of Dermatology*, **113**, 592.
- LERNER, A.B., DENTON, C.R. & FITZPATRICK, T.B. (1953) Clinical and experimental studies with 8-methoxypsoralen in vitiligo. *Journal of Investigative Dermatology*, **20**, 299.
- LIDEN, S. & MICHAELSSON, G. (1974) Dithranol (anthralin) in psoriasis. The effect on DNA synthesis, granular layer, and parakeratosis. *British Journal of Dermatology*, **91**, 447.
- LOWE, N. (1977) Essential fatty acid deficient hairless mouse: The effects of topical agents on the epidermis. *British Journal of Dermatology*, **97**, 39.
- OBERMAYER, M.E. & BECKER, S.W. (1935) A study of crude coal tar and allied substances. *Archives of Dermatology and Syphilology*, **31**, 796.
- PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, L. & PATHAK, M. (1974) Photochemotherapy of psoriasis with oral methoxsalen and long wavelength ultraviolet light. *New England Journal of Medicine*, **291**, 1207.
- PATHAK, M.A. & BISWAS, R.K. (1977) Skin photosensitizing and DNA cross linking ability of photochemotherapeutic agents. *Journal of Investigative Dermatology*, **68**, 236.
- SWANBECK, G. & LIDEN, S. (1966) The inhibitory effect of dithranol (anthralin) on DNA synthesis. *Acta dermato-venereologica*, **46**, 228.
- TANENBAUM, L., PARRISH, J.A., PATHAK, M.A., ANDERSON, R.R. & FITZPATRICK, T.B. (1975) Tar phototoxicity and phototherapy for psoriasis. *Archives of Dermatology*, **111**, 467.
- WALTER, J.F., VOORHEES, J.J., KELSEY, W.H. & DUELL, E.A. (1973) Psoralen plus black light inhibits epidermal DNA synthesis. *Archives of Dermatology*, **107**, 86.
- WALTER, J.F. & VOORHEES, J.J. (1973) Psoriasis improved by psoralen and black light. *Acta dermato-venereologica*, **53**, 469.
- WEBER, G. (1974) Combined 8-methoxypsoralen and black light therapy of psoriasis. *British Journal of Dermatology*, **90**, 317.

Case Reports

Pemphigus vulgaris and thymoma. A T-lymphocyte defect?

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Krain (1974) reviewed 8 cases of pemphigus associated with thymoma, benign or malignant, and Lynfield, Pertshuck & Zimmerman (1973) a patient with contact dermatitis and pemphigus 13 years after removal of thymoma. There are numerous reports of thymoma connected with other immunological diseases such as myasthenia gravis, systemic lupus erythematosus, erythroid aplasia and even fungal infections (Larsson, 1963; Kough & Barnes, 1964; Burstein, 1974).

CASE REPORT

A 55-year-old man was admitted in December 1975 for a widespread bullous disease. A skin biopsy specimen revealed a suprabasal acantholytic bulla; direct immunofluorescence microscopy showed a deposit of anti IgG and anti C3 in the basal epidermal intercellular region and the patient's serum contained antibodies against intercellular epidermal antigen, findings consistent with pemphigus vulgaris. The anti-nuclear factor titre was 250. Treatment was started with prednisone 110 mg a day, increasing to 250 mg, and azathioprine 150 mg daily. While on 40 mg of prednisone (April 1976) he developed a right bundle branch block and died. Autopsy revealed emboli of the great arteries of the lungs and a histologically benign thymoma weighing 112 g.

DISCUSSION

Michlmayr *et al.* (1976) have demonstrated a lymphocytotoxic antibody against the T cell in 22 of 24 patients with systemic lupus erythematosus. Guearra, Reis & Guearra (1976) found a decrease in the total T cell count in 30 patients with South American pemphigus foliaceus and a depletion of T cells in the paracortical areas of the lymph nodes. This may indicate that postnatal thymitis or thymoma may cause failure of the T cell suppressor function. It is postulated that a subsequent proliferation of 'forbidden' B cells might then, in our case, have produced antibodies against epidermal intercellular substance.

REFERENCES

- BURSTEIN, H.J. (1974) Chronic mucocutaneous candidiasis, thymoma and myopathy: a triad. *Cutis*, **13**, 262.
GUEARRA, H. DE A., REIS, A.P. & GUEARRA, M.V. (1976) T and B lymphocytes in South American pemphigus foliaceus. *Clinical and Experimental Immunology*, **23**, 477.

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- KOUGH, R.H. & BARNES, W.T. (1964) Thymoma associated with erythoid aplasia, bullous skin eruption and the LE cell phenomenon. *Annals of Internal Medicine*, **61**, 308.
- KRAIN, L.S. (1974) The association of pemphigus with thymoma, a critical review. *British Journal of Dermatology*, **90**, 397.
- LARSSON, O. (1963) Thymoma and systemic lupus erythematosus in the same patient. *Lancet*, **ii**, 665.
- LYNFIELD, Y.L., PERTSHUK, L.P. & ZIMMERMAN, A. (1973) Pemphigus erythematosus provoked by allergic contact dermatitis. *Archives of Dermatology*, **108**, 696.
- MICHLMAYR, G., PATHOULI, C., HUBER, C. & HUBER, H. (1976) Antibodies for T lymphocytes in systemic lupus erythematosus. *Clinical and Experimental Immunology*, **24**, 18.

Erythroderma, an unusual manifestation of B cell lymphoma

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SUMMARY

A case is described in which the initial manifestation of a malignant lymphoma was an erythematous skin rash over the face, scalp and trunk. Immunological surface marker analysis showed the malignant cells to be of B lymphocyte origin with a homogeneous secretion of IgM- κ . Repeated skin and lymph node biopsies and finally post mortem documented the progression of the lymphoma from that of a mixed lymphocytic-histiocytic type with a nodular pattern of growth to a diffuse histiocytic lymphoma with frequently bizarre cells. The theory of ecotaxis or homing of lymphocytes to the skin and other non-lymphoid organs of the body is discussed in an attempt to explain the occurrence of cutaneous involvement as a primary manifestation of lymphoma.

Malignant B cell lymphomas presenting as primary involvement of the skin are uncommon. The majority of such cases occur as single nodules on the scalp, face or neck (Long *et al.*, 1976). Multiple nodules and plaques are uncommon modes of presentation. Mycosis fungoides and Sézary syndrome, both lymphoproliferative malignancies of T cell origin, on the other hand, usually manifest as scaly eruptions which progress to plaques, tumours and ulcers, or as a generalized pruritic erythroderma which may be exfoliative (Winkelman & Linman, 1973; Lutzner *et al.*, 1975).

In the present report we describe a patient who presented with a diffuse erythroderma 3 years before extracutaneous lymphoma was diagnosed. Surface marker studies characterized the lymphoma to be of B cell origin and repeated biopsies and finally autopsy documented the morphological progression of the malignancy.

CASE HISTORY

D.H., a Caucasian male, born in 1944, developed over a period of 2 weeks in 1972, an extensive, raised and oedematous rash of the face, scalp and trunk which was mildly pruritic. Following histological examination, this was diagnosed as lupus erythematosus profundus and treated with chloroquine.

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TABLE 1. Surface markers of circulating lymphocytes

	% of total lymphocyte counts				
	Feb. 1975	June 1975	Aug. 1975	Aug. 1975	July 1977
E	33	68	68	71	46
M		N.D.	N.D.	N.D.	6
Ig polyvalent	28	1.5	6	23	36
IgG	21	0	10	9	12
IgA	3	0	2	2	1
IgM	3	0.5	5	10	6
IgD		N.D.	N.D.	N.D.	0
IgE		N.D.	3	1	N.D.
Ig κ					31
Ig λ					0
Blood lymphocyte count/ μ l	1530	1650			740

Initial response to therapy was good but the rash recurred and was treated with prednisolone and azathioprine with little benefit.

The patient first presented to The Queen Elizabeth Hospital on 14.1.75. His rash was then associated with bilateral enlargement of cervical and axillary lymph nodes. Relevant investigations showed a leukocyte count of $3500/\text{mm}^3$ (neutrophils 62%, lymphocytes 26%, monocytes 9%, eosinophils 3%), ESR 13 mm/h, and the LE cells, antinuclear factor and autoantibodies were all negative. Serum



FIGURE 1. An extensive indurated erythematous rash is present over the face, scalp and trunk.

creatinine was 0.124 mmol/l and blood urea 9.5 mmol/l. A renal biopsy showed no significant abnormality. Biopsy of a supraclavicular lymph node was interpreted as showing reactive hyperplasia. Results of surface marker analysis of circulating lymphocytes are presented in Table 1.

A liver biopsy performed for hepatomegaly, detected in July 1975, showed an infiltration by small lymphoid cells and histiocytes suggesting the possibility of a lymphomatous infiltrate. Clinical examination revealed moderate enlargement of the spleen. In addition, there was cardiomegaly, bilateral pulmonary consolidation, pleural effusions and the extensive rash was still present over the face, scalp and trunk (Fig. 1). At this time the toxoplasma immunofluorescent antibody titre was discovered to be 1/256 and complement fixation test was positive at 1/512 dilution. Stored sera from 3 months previously were negative for toxoplasma antibody. He was treated with pyrimethamine and sulphadimidine for toxoplasmosis. At this admission, a skin biopsy was taken from the erythematous areas on the chest and a cervical lymph node was submitted for histological examination.

HISTOLOGY

The lymph node was completely replaced by small lymphoma cells which displayed irregular nuclei with compact chromatin and inconspicuous nucleoli. Nuclear cleavage was evident and the cytoplasm was scanty (Fig. 2). Mitotic figures were rare. These cells were aggregated into a nodular pattern (Fig. 3) but the cleaved cells were also seen in between the nodules. A second cell-type was present but they formed less than 25% of the total population. These were non-cleaved cells with moderate amounts of cytoplasm and they had vesicular nuclei which contained one or two large nucleoli. Also present in the biopsy were large epithelioid cells scattered throughout the lymph node and rarely within the subcapsular sinus. These cells were interpreted as part of the Piringer-Kuchinka response

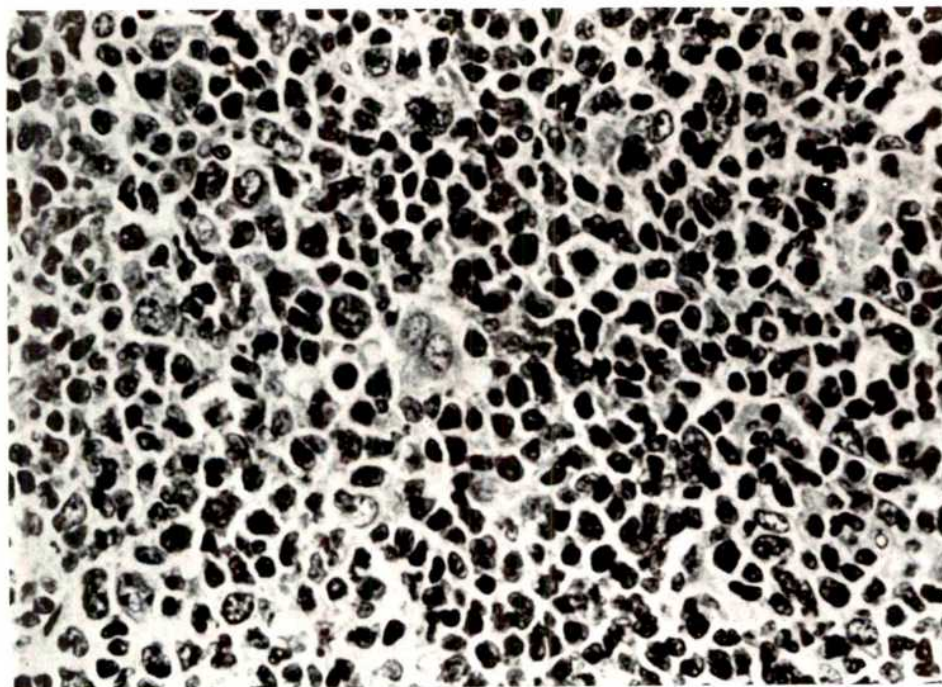


FIGURE 2. Lymph node. Lymphocytes with irregular cleaved nuclei are present together with some non-cleaved cells (H & E, original magnification $\times 300$).

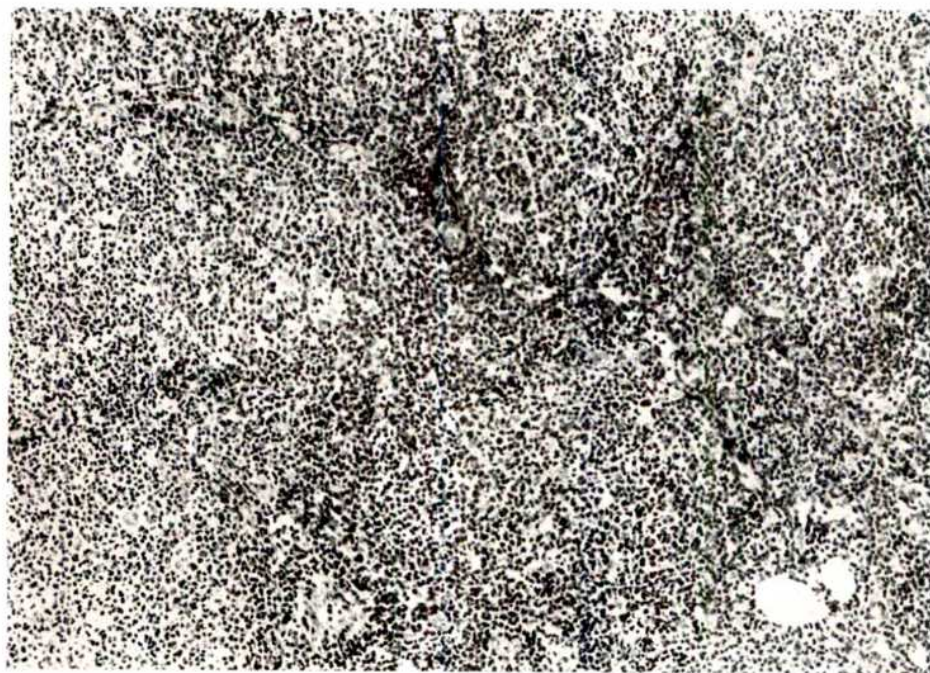


FIGURE 3. Lymph node. The nodular pattern of the lymphoma is evident (H & E, original magnification $\times 125$).

to toxoplasmosis (Leong *et al.*, 1975) and the patient had corresponding raised levels of toxoplasma antibodies.

The skin biopsy showed nodular cellular infiltrates within the deep dermis which were aggregated around dermal appendages and blood vessels with extension into the subcutaneous tissue (Fig. 4). The cells were predominantly of the cleaved type similar to those seen in the lymph node. Non-cleaved cells were also present. The overlying epidermis was not involved and the papillary dermis was spared. Pautrier microabscesses were not present in the epidermis and cells with convoluted nuclei were not a feature of the lymphomatous infiltrate. At this stage, the initial skin biopsy of 1972 and the supraclavicular lymph node biopsy of January 1975 were reviewed. Both biopsies examined in retrospect were considered to show a nodular lymphoma of mixed lymphocytic-histiocytic cell type. The material was also reviewed in consultation by Dr R.F. Dorfman of Stanford University Medical Center who concurred with the diagnoses.

A skin biopsy performed a month later showed similar features but of a greater severity. The nodular infiltrates in the deep dermis were frequently confluent; however, the epidermis and papillary dermis remained uninvolved. A bone marrow biopsy was not involved.

Intensive chemotherapy was commenced in September 1975. He received cyclophosphamide, vincristine and prednisolone, and subsequently combination regimes containing hydroxydaunorubicin and bleomycin. At best, the disease was moderately controlled. Cardiomegaly resolved after treatment for toxoplasmosis.

In June, 1977, a cervical lymph node and the adjacent skin were biopsied. On microscopic examination, the cutaneous infiltrate was found to be more extensive and the deep dermal nodules were frequently confluent. The lymph node showed a nodular infiltrate of both cleaved and non-cleaved cells but this time the non-cleaved cells were in predominance and mitotic figures were present in

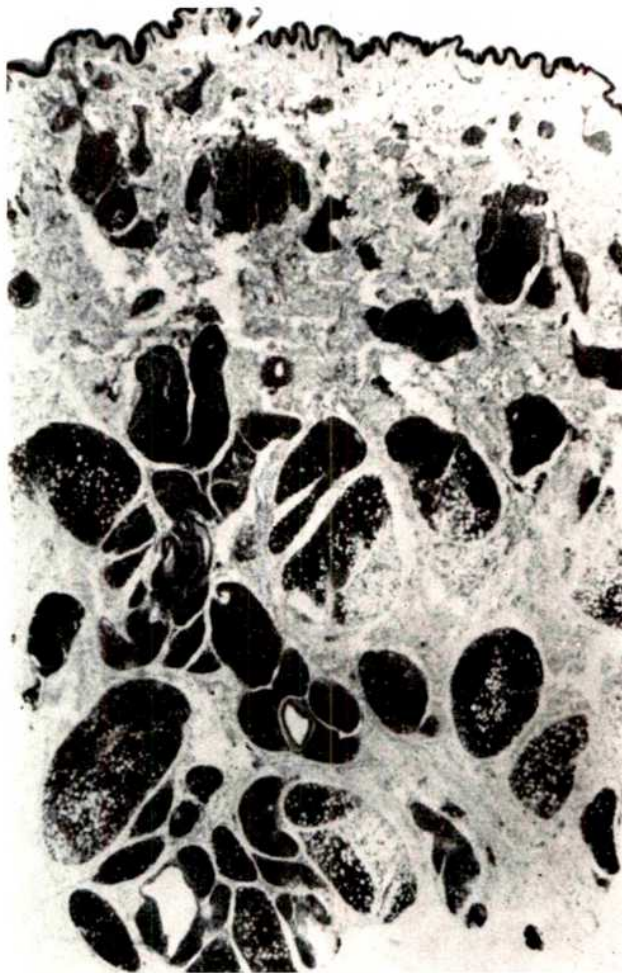


FIGURE 4. Nodular infiltrate of lymphoma present in the deep dermis and subcutaneous tissue. The superficial dermis and epidermis are uninvolved (H & E, original magnification $\times 3$).

increased numbers. Lymphocytes were extracted from both skin and lymph node biopsies and studied for immunological surface markers (Forbes & Zalewski, 1975). A monotypic infiltration of B lymphocytes with IgM- κ surface immunoglobulin was demonstrated (Table 2). Bone marrow biopsy revealed infiltration by a lymphoma of mixed cellularity.

Immunological function studies in March, June and August 1975 showed depression of PHA responsiveness and failure of tetanus antibody response. Serum immunoglobulin values were in the normal range (IgG 15.0 g/l, IgA 0.3 g/l, IgM 1.1 g/l). A satisfactory response was obtained to typhoid immunization.

The patient presented terminally in August 1977 with pulmonary consolidation and bilateral pleural effusions. Cells from the pleural effusions were analysed for surface markers and the majority were found to be small lymphocytes with characteristics of T lymphocytes. A smaller number of lymphocytes had surface immunoglobulin but a distinctly abnormal group of larger cells with vacuolated cytoplasm was present. These had the characteristics of the lymphoma cells seen in the skin

TABLE 2. Surface markers of lymph node and skin nodule lymphocytes

	% of cells		
	Lymph node	Skin nodule	Pleural effusion
E	27.5*	9*	93*
M	5.5	3	6
Ig polyvalent	67	86.5	9
IgG	0	0	4
IgA	0	0	0
IgM	72	88	7
IgD	0	0	0
κ	70.5	91	14
λ	0	0	2

* The majority of E rosetting cells were small lymphocytes. Lymph node and skin nodule analyses were done in June 1977 and pleural fluid in July 1977. The surface Ig positive cells in the pleural effusion were distinctly larger cells with prominent nucleoli and vacuolated cytoplasm.

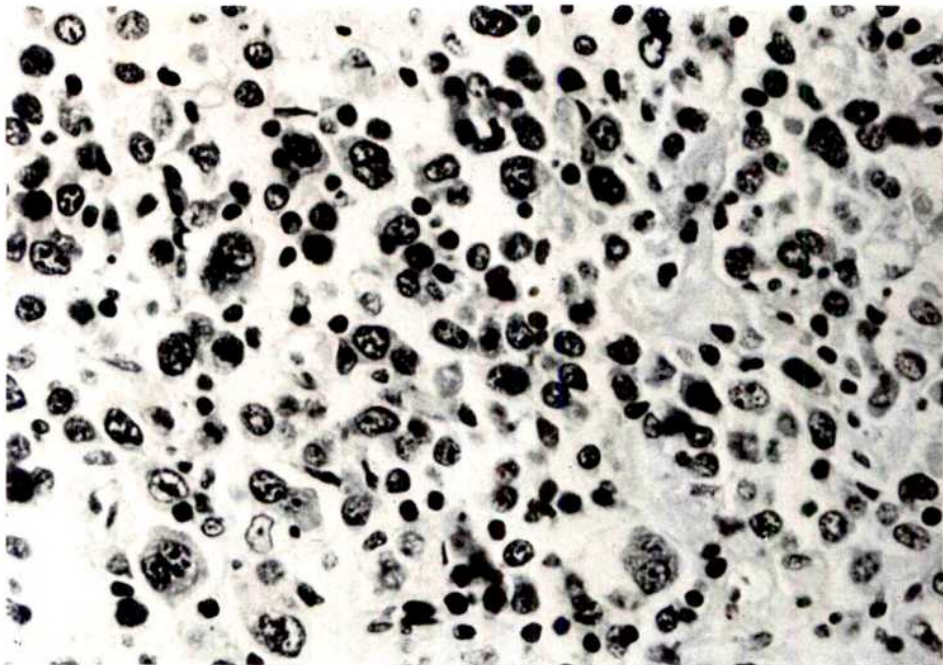


FIGURE 5. Lymph node at autopsy showing a diffuse infiltrate of non-cleaved cells with frequent multinucleated forms (H & E, original magnification $\times 300$).

and lymph nodes. The results (Table 2) were interpreted as showing primarily an inflammatory exudate together with some lymphoma cells.

AUTOPSY FINDINGS

At autopsy, the indurated erythematous skin rash was found over the scalp, face and extensively over the chest and the shoulders. Pink-tan nodules and plaques were present on the pleura and pericardium including a solitary deposit in the epicardium of the anterior left ventricle. There was enlargement of all subcutaneous and visceral lymph nodes and the peritoneal and pelvic cavities were extensively infiltrated by tumour. Tumour deposits were present in the liver, kidneys and in the vertebral bodies. Histological examination revealed a distinct change in the morphology of the malignant cells. These showed considerable pleomorphism and were poorly cohesive. They were all large non-cleaved cells with moderate amounts of cytoplasm and their nuclei were vesicular and contained large eosinophilic nucleoli (Fig. 5). Giant bizarre multinucleated forms were frequent. Mitotic activity was brisk and the pattern of infiltration was diffuse with no semblance of nodularity.

DISCUSSION

Mycosis fungoides and Sézary syndrome present as pruritic erythroderma. These two diseases are now recognized as lymphoproliferative malignancies of T cell origin (Lutzner *et al.*, 1975). The other primary malignant lymphomas of skin, the majority of which are of B cell origin, present more frequently as single non-ulcerated cutaneous nodules on the scalp, face and neck. In the series of 25 cases of primary lymphomas of the skin reported by Long *et al.* (1976), involvement of the extremities occurred in 40% of cases but multiple nodules or plaques were uncommon modes of presentation and an erythematous rash did not occur in any of their patients. The presentation of a diffuse, mildly pruritic erythematous rash which mimicked that of mycosis fungoides and Sézary syndrome does not appear to have been reported previously in cutaneous lymphoma of B cell origin.

It is said that pruritus is a universal symptom in the cutaneous T cell lymphomas (Winkelman, 1974). In these cases the rash is scaly or exfoliative corresponding to the microscopic finding of exocytosis of the lymphoma cells into the epidermis, forming micro-abscesses or even producing a spongy disaggregation of the epidermal cells. In the case of the B cell lymphomas, however, there is preservation of the epidermis and a distinct uninvolved Grenz zone of papillary dermis separates the infiltrate from the epidermis (Burg *et al.*, 1977). The lymphomatous infiltrate frequently involves the deep dermis and may invade the subcutaneous tissue as in our patient. Furthermore, the absence of atypical cells with convoluted nuclei histologically helps distinguish cutaneous B cell lymphomas from those of T cell origin.

The demonstration of a homogenous surface immunoglobulin restricted to the IgM- κ class indicated the monoclonal nature of the lymphoma cells in our patient and confirmed the value of surface marker analysis in the diagnosis of malignant lymphoid infiltrates.

Another interesting feature noted in our case was the change in the morphology of the lymphomatous infiltrate through the course of the disease. The skin and the first lymph node biopsy showed a mixed lymphocytic-histiocytic lymphoma (Rappaport, 1966) distributed in a nodular or follicular pattern. Interfollicular extension was noted in the lymph node. Two years later, the composition of the lymphoma had changed to one of non-cleaved or 'histiocytic' predominance retaining an identifiable nodular pattern. The skin showed a similar change in cellular composition of the tumour infiltrates which tended to be confluent in the deep dermis. At autopsy, the tumour infiltrates were all diffuse and composed purely of large non-cleaved cells or 'histiocytes' with prominent nucleoli. Frequent giant and bizarre forms were present, many being multi-nucleated. Such a change in

morphology has been observed previously in the aggressive phase of the disease and in tissue examined at autopsy (Lukes & Collins, 1975).

In a recent report, Long *et al.* (1976) reiterated that malignant lymphoma can present primarily in the skin. In their patients the interval from apparently localized cutaneous lesions to involvement of lymph nodes and/or viscera ranged from 6 months to 5 years. Their findings indicated that malignant lymphomas with primarily skin manifestation almost invariably disseminate to extracutaneous organs with a usually fatal outcome. This was true with our patient who presented with skin infiltration 3 years before lymph node involvement was diagnosed and at autopsy almost 6 years later there was extensive lymph node as well as visceral dissemination. The occurrence of malignant lymphoma as a primary cutaneous manifestation raises the fundamental question of what determines the localization of malignant lymphocytes to skin or other extra nodal tissue.

In their attempt to explain the 'ecotaxis' (De Sousa, 1971) or 'homing' of lymphocytic malignancies to non-lymphoid tissues, Goudie *et al.* (1974) suggested that small numbers of circulating lymphocytes may home on quite sharply defined areas of the body not usually regarded as secondary lymphoid organs. This principle was adduced to explain the symmetrical deposition of lymphomatous lesions which have been described in such organs as the breast (Goudie *et al.*, 1974), conjunctiva (Morgan, 1971), and testes (Tellem *et al.*, 1961). In the context of our patient, the disposition of primary cutaneous lymphoma can be explained on the basis of the emergence of a single clone of neoplastic lymphocytes with ecotaxis of a highly tissue-specific nature.

ACKNOWLEDGMENTS

We wish to record our appreciation to Dr R.F. Dorfman of Stanford University Medical Center for reviewing the material.

REFERENCES

- BURG, G., RODT, H., GROSSE-WILDE, H., NETZEL, B., FATEH MOGHADAM, A. & BRAUN-FALCO, O. (1976) Enzyme-cytochemical and immunocytological studies as a basis for the revaluation of the histology of cutaneous B cell and T cell lymphomas. In: *Immunological Diagnosis of Leukaemia and Lymphomas* (Ed. by S. Thierfelder, H. Rodt and E. Thiel), p. 179. Springer-Verlag, Berlin.
- DE SOUSA, M. (1971) Kinetics of the distribution of thymus and marrow cells in the peripheral lymphoid organs of the mouse: Ecotaxis. *Clinical and Experimental Immunology*, **9**, 371.
- FORBES, I.J. & ZALEWSKI, P.D. (1976) A subpopulation of human B lymphocytes that rosette with mouse erythrocytes. *Clinical and Experimental Immunology*, **26**, 99.
- GOUDIE, R.B., MACFARLANE, P.S. & LINDSAY, M.K. (1974) Homing of lymphocytes to non-lymphoid tissues. *Lancet*, **i**, 292.
- LEONG, A.S.Y., WANG, F., THOMAS, V. & ONG, T.H. (1976) Acquired toxoplasmosis in Malaysia. *South East Asian Journal of Tropical Medicine and Public Health*, **7**, 10.
- LONG, J.C., MIHM, M.C. & QAZI, R. (1976) Malignant lymphoma of the skin. A clinicopathologic study of lymphomas other than mycosis fungoides diagnosed by skin biopsy. *Cancer*, **38**, 1282.
- LUKES, R.J. & COLLINS, R.D. (1975) New approaches to the classification of the lymphomata. *British Journal of Cancer*, **31**, Suppl. **II**, 1.
- LUTZNER, M., EDELSON, R., SCHEIN, P., P. GREEN, I., KIRKPATRICK, C. & AHMED, A. (1975) Cutaneous T cell lymphomas: The Sézary syndrome, mycosis fungoides and related disorders. *Annals of Internal Medicine*, **83**, 531.
- MORGAN, G. (1971) Lymphocytic tumours of the conjunctiva. *Journal of Clinical Pathology*, **24**, 585.
- RAPPAPORT, H. (1966) Tumours of the haematopoietic system. In: *Atlas of Tumour Pathology*, p. 97. Armed Forces Institute of Pathology, Washington, D.C.
- TELLEM, M., FALK, A. & MERANZE, D.R. (1961) Bilateral malignant lymphoma of the testes. *Archives of Pathology*, **71**, 151.
- WINKELMANN, R.K. (1974) Clinical studies of T-cell erythroderma in Sézary syndrome. *Mayo Clinic Proceedings*, **49**, 519.
- WINKELMANN, R.K. & LINMAN, J.W. (1973) Erythroderma with a typical lymphocytes (Sézary syndrome). *American Journal of Medicine*, **55**, 192.

Comment

**A THEORETICAL NOTE ON THE TOPOLOGICAL
FEATURES OF MELANIN TRANSFER**

Some years ago Daniels (1963) drew attention to the fact that the predominant distribution of pigment in the basal layers of the epidermis was inconsistent with the generally accepted proposal that the function of melanin is to act as a light-absorbing filter. I have argued (Riley, 1974) that the facility of free radical generation in melanin when irradiated with photons of sufficient quantal energy may have biological significance in that free-radical-initiated cytoplasmic damage, such as lipid peroxidation, would be ensured in those cells exposed to radiation of sufficient intensity to cause potentially deleterious mutations, with the consequent elimination of mutant cells from the generative population. This would explain the basal supranuclear disposition of epidermal melanin. McGinness and his colleagues (Proctor, McGinness & Corry, 1974) have gone further and suggested that destruction of melanized cells can be brought about by non-radiative energy transfer with melanin acting as an amorphous semiconductor.

It has previously been suggested (Slater & Riley, 1966) that the free radical properties of melanin necessitate encapsulation of the pigment in order to protect the cells responsible for its synthesis. If such protection is successful in melanocytes it is necessary to furnish an explanation of the subsequent sensitization of keratocytes to which the pigment is transferred.

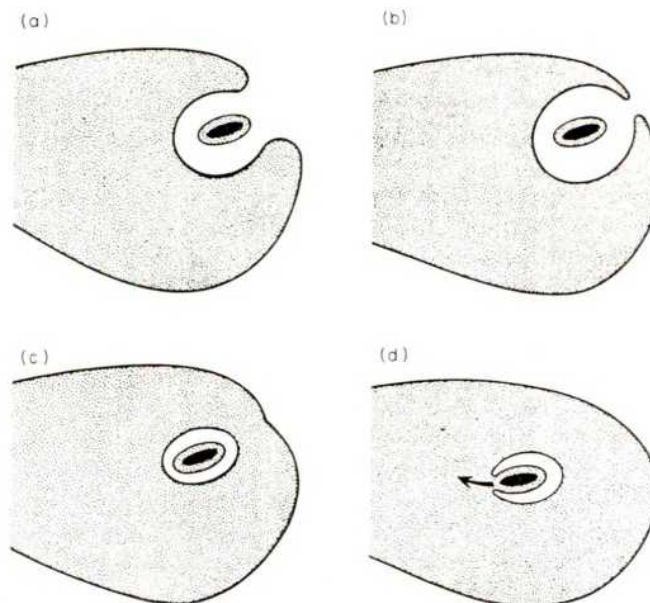


FIGURE 1. Schematic outline of stages in phagocytosis of a melanin granule. The granule is composed of a melanin-coated matrix (black) encapsulated in a membrane. The internalized phagocytotic vesicle (c) may discharge the melanin directly into the cytosol of the recipient cell (d) if fusion of the membranes takes place. The stippling indicates topological identity.

One approach to an explanation of this difference in sensitivity concerns the topological relationship between the melanin granule and the membrane systems of the donor and recipient cells. The simplest case may be envisaged schematically as in Fig. 1. An isolated melanin granule, coated with a single layer of membrane, is phagocytosed with the formation of a phagosome (Fig. 1c) in which the melanin granule is surrounded by two layers of membrane. It may be important to note that the melanin is topographically *outside* the membrane so that the apposed membrane layers have the same polarity. Assuming that there is no impediment to the fusion of these membranes, direct continuity would be established between the melanin and the cytosol of the recipient cell (Fig. 1d). Whether this occurs in melanin transfer in the skin is dubious, although it is possible that such a phenomenon could occur under other conditions, e.g. as in the case of melanin phagocytosed by macrophages in the experiment of Johnson, Mandel & Daniels (1972).

A possible variant of melanin transfer is illustrated in Fig. 2. Compound melanosomes, which may be the product of autophagy, would give rise to four layers of membrane (Fig. 2c) if transferred by the 'pinching-off' mode of cytoplasmic transfer referred to below. However, this process appears to be relatively rare and can be shown to be topologically equivalent to the case in Fig. 1.

The generally accepted mechanism of melanin transfer from melanocytes to keratocytes is schematically outlined in Fig. 3. This shows the 'pinching-off' of melanocyte cytoplasm containing melanosomes. Subsequent fusion of the phagosome membrane with the enclosed melanocyte-derived plasma membrane would result in the release of membrane-coated melanin granules (i.e. melanosomes) into the keratocyte cytoplasm. This may allow direct fusion of keratocyte lysosomes with melanosomes

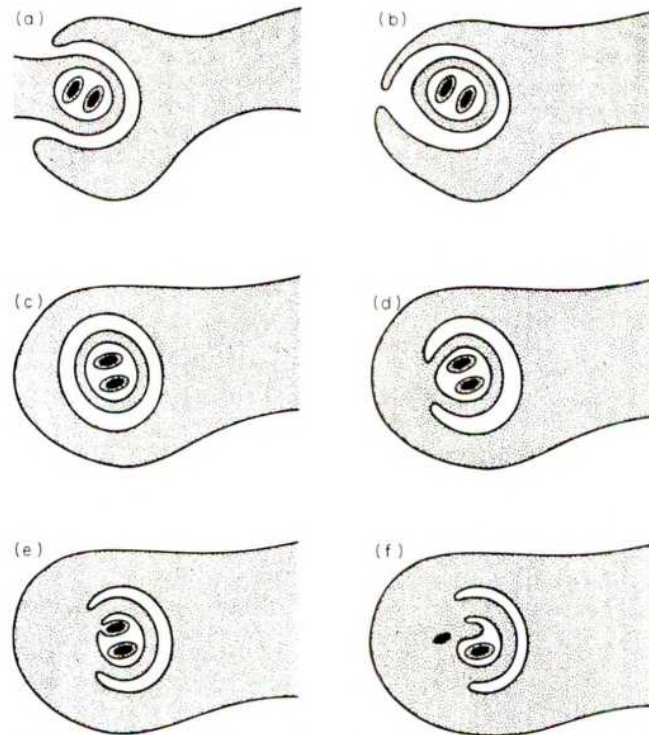


FIGURE 2. Sequence of stages in melanin transfer by phagocytosis of the tip of a dendrite containing a compound melanosome. The membrane layers have opposing polarities and, on fusion, would discharge melanin into the recipient cytosol (Stage f).

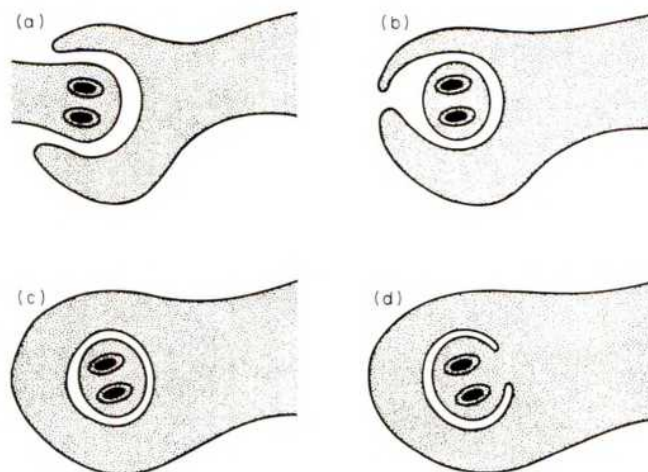


FIGURE 3. Outline of melanin transfer by dendrite phagocytosis ('pinching off') showing that fusion of the apposed membrane layers of the phagocytic vacuole (c) leaves a residual layer of membrane surrounding the melanin.

with the result that damage to the 'melanolysome' membrane by melanin free radical-initiated reactions, such as lipid peroxidation, will result in the release of hydrolytic enzymes and widespread cellular damage. This process is closely analogous to the sequence of events proposed for cellular damage in other types of photosensitization (Slater & Riley, 1966; Slater, 1972).

From a topological standpoint the opportunities for fusion between melanosomes and lysosomes in keratocytes appear to be identical to those existing in melanocytes and, therefore, the difference between these cell types in their susceptibility to damage by irradiation demands a further explanation. Melanocytes possess a very efficient free radical quenching mechanism which apparently operates by the formation of glutathione adducts giving rise to cysteinyl conjugates in the case of melanin precursors (Rorsman, 1974), and the significance of the cytotoxic effects on melanocytes produced by compounds which may limit glutathione levels (Frenk *et al.*, 1968) cannot be overlooked. On the other hand, it is probable that keratocytes possess a larger number of lysosomes so that the statistical chances of the collision and fusion of lysosomes and melanosomes to give rise to a 'melano-lysosome' are greater in keratocytes than in melanocytes. It is likely that both of these factors contribute to the difference in the response of these cells to radiation but as yet the relative importance of free radical quenching or the rarity of melanocyte lysosomes has not been established.

P.A.RILEY

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REFERENCES

- DANIELS, F., JR (1963) Ultraviolet carcinogenesis in man. *National Cancer Institute Monographs*, **10**, 407.
FRENK, E., PATHAK, M.A., SZABO, G. & FITZPATRICK, T.B. (1968) Selective action of mercaptoethylamines on melanocytes in mammalian skin. *Journal of Investigative Dermatology*, **18**, 119.

- JOHNSON, B.E., MANDEL, G. & DANIELS, F., JR (1972) Melanin and cellular reactions to ultraviolet radiation. *Nature*, **235**, 147.
- PROCTOR, P., MCGINNESS, J. & CORRY, P. (1974) A hypothesis on the preferential destruction of melanised tissues. *Journal of Theoretical Biology*, **48**, 19.
- RILEY, P.A. (1974) Epidermal dendrocytes. In: *Physiology and Pathophysiology of Skin* (Ed. by A. Jarrett), Vol. 3, pp. 1101-1235. Academic Press, London.
- RORSMAN, H. (1974) The melanocyte illuminated. *Transactions of the St John's Hospital Dermatological Society*, **60**, 135.
- SLATER, T.F. & RILEY, P.A. (1966) Photosensitization and lysosomal damage. *Nature*, **209**, 151.
- SLATER, T.F. (1972) *Free Radical Mechanisms in Tissue Injury*. Pion Ltd, London.

Obituary

DR F.J.O'DONNELL

Frank O'Donnell, who died suddenly in January last, was born in 1894 and was in practice until the day before his death. Surely the doyen of practising Dublin doctors and perhaps the last of a vanishing race of general physician-dermatologists.

Graduating from the Schools of Surgery of the Royal College of Surgeons in Ireland in 1920 he spent some years in England and in 1925 returned to Dublin as Medical Officer to the Rathfarnham Dispensary District. In 1925 he was elected a Fellow of the Royal College of Physicians of Ireland having taken his membership in 1923. He was later to become President of the College from 1953 to 1958.

Appointed Assistant Physician to Mercers Hospital in 1928 he concentrated his interests and energies upon Dermatology and a few years later was elected Dermatologist to the City of Dublin Skin and Cancer Hospital. He was a member of both the British and the Irish Associations of Dermatologists and of the North of England Dermatological Society. He was a Director and ex-Governor of the Apothecaries Hall of Dublin.

As long as his physical powers allowed he travelled regularly to attend dermatological meetings. Indeed his continuing interest and critical enthusiasm were for long an example to his juniors. Stubborn and difficult in negotiations as some may have found him, his personal friendliness and wit made him a welcome member of many less contentious medical groups.

His wife died some years ago. They had no children.

DAVID M. MITCHELL

Correspondence

Vitiligo and leprosy

SIR, I would like to put on record the observation that, during my work as a leprologist in London, I have encountered a significant incidence of vitiligo in patients under treatment for lepromatous leprosy. By 'vitiligo' I am not referring to the well known hypopigmentation of leprosy but to the classical circumscribed depigmentation that can affect healthy persons of all races but occasionally is found in association with organ-specific auto-immune disorders such as diabetes, pernicious anaemia, thyroid disease, Addison's disease, and alopecia areata. I have found 8 cases of vitiligo among 114 lepromatous patients, an incidence of 7%, but no cases of vitiligo among a larger number of non-lepromatous patients, and I would be interested to know if this association has been noted elsewhere or if it has been reported in the literature.

This observation, when fully investigated by my successor at the Hospital for Tropical Diseases, may lend support to the hypothesis that vitiligo is an auto-immune disorder, having regard to the wide variety of circulating auto-antibodies which have been described in lepromatous leprosy, such as anti-nuclear, antithyroid and antisperm antibodies, and rheumatoid factor.

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W.H. JOPLING

Book Reviews

Struktur und Pathologie des Hautnervensystems. Edited by G.LASSMANN, W.JURECKA and G.NIEBAUER (1977) Vienna: Verbandes der Wissenschaftlichen Gesellschaften Österreichs. Price Sch 525, \$35.00.

Any new work on the pathology of the cutaneous nervous system is timely because, apart from a few notable exceptions, little of importance in this field appears to have emerged during the past few years. The reasons for this shortcoming are obviously complex; and not least among them are the difficulties in obtaining satisfactory preparations for microscopical analysis. Furthermore, interpretations of apparent pathological alterations in cutaneous nerves are usually restricted to those conditions demonstrating gross deviation from the norm, whilst the more subtle and possibly more important changes may pass undetected. This book goes some of the way in illustrating these difficulties and shows how newer techniques, including electron microscopy and fluorescence microscopy, may be usefully applied to the examination of skin nerves in health and disease.

This paper-back volume contains the proceedings of a symposium on the pathology of the cutaneous nervous system, which was held in Vienna in April 1977. Its 38 papers cover a wide range of neuropathological conditions as encountered by dermatologists. All articles are in German, except for three in English. Those readers not fluent in German, therefore, will find difficulty in understanding much of the work, although an English summary is provided in most instances.

The first paper is a brief account (in English) by Breathnach on the development of cutaneous nerves, illustrated by a selection of elegant electron micrographs of freeze-fractured and 'thin-sectioned' material. Lassmann then reviews special methods for studying cutaneous nerves by light microscopy and demonstrates the particular value of fluorescence microscopy and enzyme histochemistry. Hartschuh and Weibe report succinctly on how studies in experimental degeneration have cast new light on Merkel cell behaviour, and Bischoff gives a useful review of sensory nerve endings in normal human skin. Di Francesco and co-workers use morphometric analytical techniques to show that no quantitative abnormality of unmyelinated nerves can be found in psoriasis. Lassmann and Gebhart discuss the value of skin biopsy in the diagnosis of metachromatic leucodystrophy. It is quite impossible to mention each paper in turn and I have thus selected a few which caught my eye.

Dermatologists with an interest in neuropathology or electron microscopy will find this book valuable, if not exactly readable or easy to digest. The photographic method of printing has obviously helped to expedite moderately rapid publication of these proceedings, but the small page size with cramped text and illustrations tends to detract from an otherwise commendable production. I found the lack of an index a particular disadvantage. The book might be seen in greater perspective and enjoyed by a wider readership if more studies on comparative anatomy and on experimental pathology had been included.

R.A.J.EADY

Dermatochirurgie in Klinik und Praxis. Edited by B.KONZ and G.BURG (1977) Berlin, Heidelberg, New York: Springer-Verlag. Pp. xii, 238. 144 illustrations.

The first German symposium on dermatosurgery took place in Munich in 1975 and this is a pleasantly produced soft-back edition of the various contributions. Professor Braun-Falco gives an introduction. Plastic, E.N.T., ophthalmic and hand surgeons as well as anaesthetists and members of burns units participated in addition to dermatologists. The difficulty for the dermatologist, also remarked on by Braun-Falco, is to recognize where his surgical limitations should lie. It is clear from their articles that some German dermatologists are excellent plastic surgeons. However, it is varicose vein surgery that apparently constitutes most of their clinical material and British dermatologists would probably not wish to enter this field. There is undoubted increased surgical scope for dermatologists in the United Kingdom, especially in outlying hospitals where there are no plastic surgeons, themselves to deal with most skin tumours, now that radiotherapy is much less favoured, as well as with axillary hyperhidrosis, rhinophyma and, perhaps, hair transplants for selected patients.

To attain the necessary skills, the Dermatological Specialist Advisory Committee to the Joint Committee on Higher Medical Training expects the trainee to obtain experience in plastic surgery, but it is also essential for members of Appointment Committees for consultant dermatologist posts to make sure the appointees possess the required ability in dermatology. Senior registrars should study German and read this book.

MICHAEL FEIWEL

Physiologie de la Circulation Cutanée. By J.P. MARTINEAUD and S. SEROUSSI (1977). Paris, New York: Masson. Pp. 116.

The introduction to this small book emphasizes the importance of the skin to the physician, dermatologist, paediatrician and physiologist.

The nine chapters, with a generous one sixth given to references, provide a useful but limited review of the physiology of the blood circulation of the skin. There has been some improvement in techniques for measurement during the last decade but a review of this kind inevitably gives most attention to 'classic' observations of the past 50 years.

Inevitably the skin is to the physiologist, as often as not, that youthful organ observed in the male members of their team. Consequently, the sections on ageing, the menstrual cycle or pathology are feeble in comparison to, for instance, the elsewhere published literature on gerontology, paediatrics or dermatology of the last decade. Haemorrheology, angiogenesis and pharmacology are important topics which like inflammation overlap with physiology but are given only the briefest mention. As might be expected in a standard physiology textbook this monograph is best on thermoregulation and the nervous control of the circulation.

T.J. RYAN

News and Notices

1st International Congress of Hair Research

This Congress promoted by the German Society of Cosmetic Chemists will be held in Hamburg, 13–16 March 1979.

Further details may be obtained from: Dralle Workshop Group, 'Scientific Hair Research', PO Box 600649, D2000 Hamburg 60, Federal Republic of Germany.

Preliminary announcement

An International Symposium on 'THE EPIDERMIS IN DISEASE' will be held at the Welsh National School of Medicine, Heath Park, Cardiff, on the 18–20 April 1979. The meeting will be divided into six sessions. The sessional Chairmen and subject of each session will be as follows:

Dr E.Beutner (USA)—'Immunopathology of epidermal disease'.

Prof. M.Greaves (London)—'Pharmacological aspects of epidermal disease'.

Prof. E.Wilson Jones (London)—'Reaction patterns of epidermal disease'.

Prof. M.Prunieras (France)—'Epidermal symbionts'.

Prof. E.Christophers (Kiel)—'Disorders characterized by increased epidermopoiesis'.

Dr R.Marks (Cardiff)—'Disorders of keratinization'.

Further details and registration forms can be obtained by contacting the co-organisers: Dr R.Marks, Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, or Prof. E.Christophers, Hautklinik der Christian-Albrechts, Schittenhelmstrasse 7, D-2300 Kiel, Germany.

The American Dermatologic Society for Allergy and Immunology announces its Third Annual Graduate Course in Basic and Clinical Immunology

The guest of honour and recipient of the Society's award this year is Dr Robert A.Good, Sloan Kettering Institute, discussing the topic, 'Immunodermatology and Cancer Therapy'. The Society also has amongst its distinguished faculty, the new president of the American Academy of Dermatology, Dr Robert Goltz who will talk on 'Skin Manifestations of the Immune Deficiency Diseases'. Other faculty members include Drs Peter Lynch, Michael Fellner, Hermann Pinkus, Aaron Lerner, and Philip Brunell.

The meeting will be held at the Airport Hilton Hotel in Denver, Colorado. The meeting is certified for 9 h of Continuing Medical Education, Category 1 Credit to the American Academy of Dermatology. The meeting will begin on Thursday morning, 28 September and end Saturday, 30 September.

Pre and post meeting activities are in the planning stage and further information can be obtained by writing to Dr Michael Fellner.

Mr and Mrs J.N.Taub International Memorial Award for Psoriasis Research, 1977

Dr Howard Green, Professor of Cell Biology, Massachusetts Institute of Technology, has been selected as the 1977 winner of the \$1000 Mr and Mrs J.N.Taub International Memorial Award for Psoriasis Research.

This Award is given to Dr Howard Green for single contributions in the field of cell and molecular biology. He originated the 3T3 fibroblast-like cell, which has been used by many researchers to unravel the molecular basis of cell proliferation. He has recently devised a method by which human keratinocytes can be serially subcultured. The availability of large quantities of human epidermal cells on a routine basis makes feasible the application of modern molecular biology to the study of those skin diseases in which the epidermis is a significant participant.

Dr Green was selected by a seven man committee, and the announcement was made by Dr John M. Knox during the Psoriasis Symposium at the Academy. The award was established in 1957 by Carol

and Henry J.N.Taub in honour of their grandparents Mr and Mrs J.N.Taub. Their establishment of this program carried with it the hope that the award would be an incentive to develop a cure to alleviate one of the problems of mankind.

University of California, San Francisco

A Postgraduate Course and Workshop in Dermatomyecology will be held in San Francisco on 25-27 August 1978.

Further information may be obtained from: Extended Programs in Medical Education, University of California, 1308-3rd Avenue, San Francisco, California 94143, U.S.A.

Appointments

South East Thames Regional Health Authority

Dr Gillian Pat Sparrow has been appointed Consultant Dermatologist to the Bromley Area Health Authority and Greenwich and Bexley Area Health Authority of the South East Thames Regional Health Authority. Dr Sparrow qualified M.B., B.Chir. from Cambridge University and St Thomas's Hospital in 1968 and became M.R.C.P. in 1971. Her post-graduate training in dermatology was at St John's Hospital for Diseases of the Skin and the Hospital for Sick Children, Great Ormond Street, London. She has a number of publications to her credit including treatment of Granuloma Annulare and Necrobiosis Lipoidica and Localized Pemphigoid.

Erratum

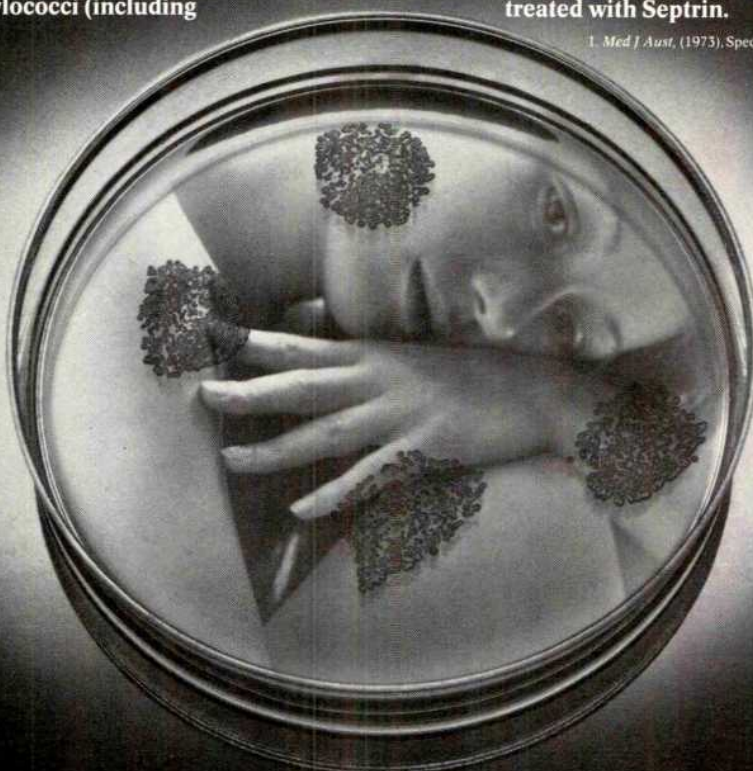
In the article by S.T.Zaynoun *et al.* 'The Treatment of Erythropoietic Protoporphyrria' (*British Journal of Dermatology*, 97, 663), the acknowledgement to Dr E.M.Donaldson should have read 'North Staffordshire Hospital Centre, Stoke-on-Trent'.

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1. Med J Aust, (1973), Special Suppl, L10



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Brit J Derm. (1971), 84, 366.

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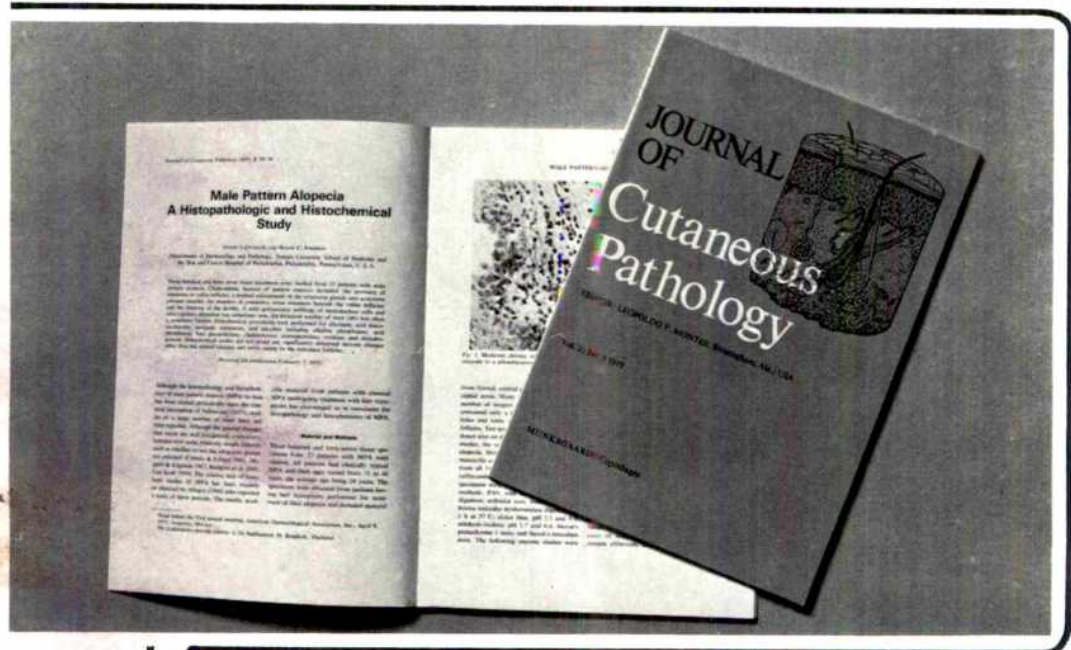


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Editor: Leopoldo F. Montes, Birmingham, Alabama

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometer(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^a 131I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 99, Number 1, July 1978

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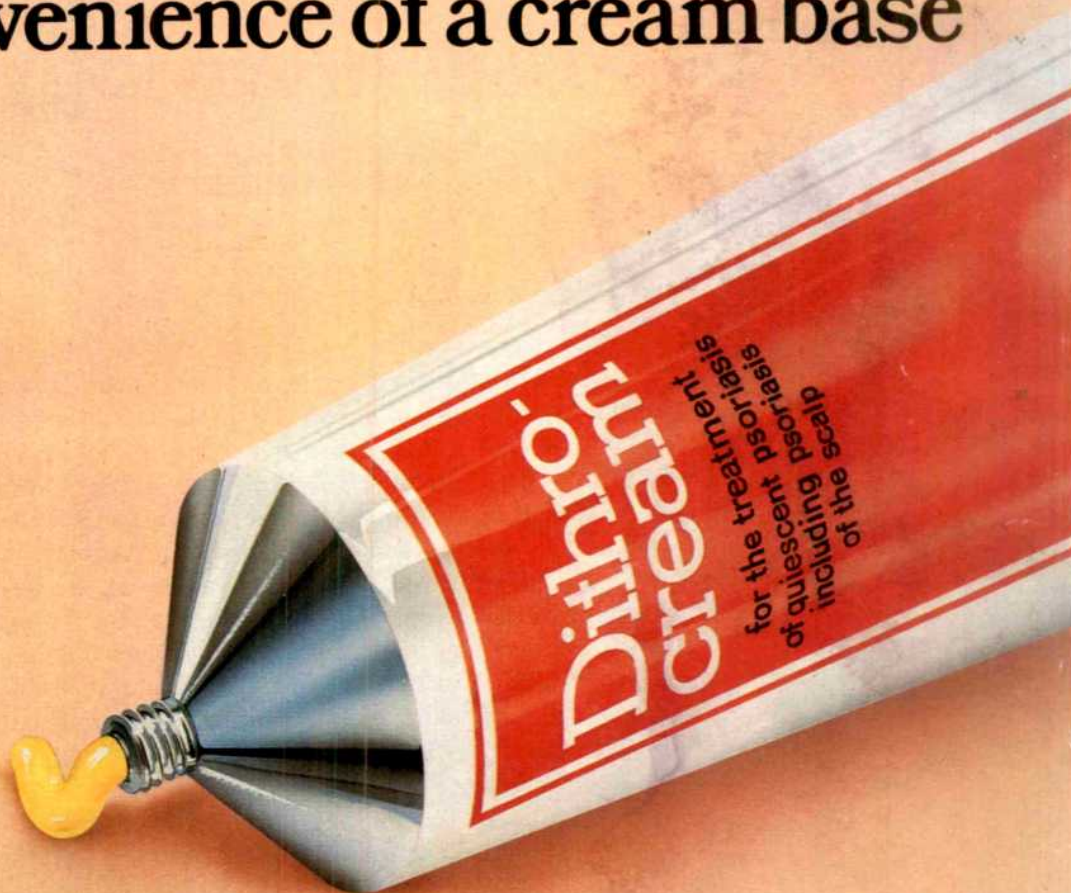
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Compatible with the 9th Revision of the
WHO International Classification of Disease

Compiled by
Suzanne Alexander and Alan B. Shrank

This new index is designed to permit the accurate and efficient retrieval of information on dermatological diagnoses, and has a much wider scope than the index issued by the World Health Organization. The coding system is based upon the International Classification of Disease, which is understood and accepted universally, and it is entirely compatible with the Ninth Revision due to be inaugurated in 1979. It is compact yet inclusive of many new disorders, and will prove its value in epidemiological studies, in medical research and in the planning of health services. The coding system is becoming widely adopted throughout the world and dermatologists will therefore find this index to be increasingly useful in their practice.

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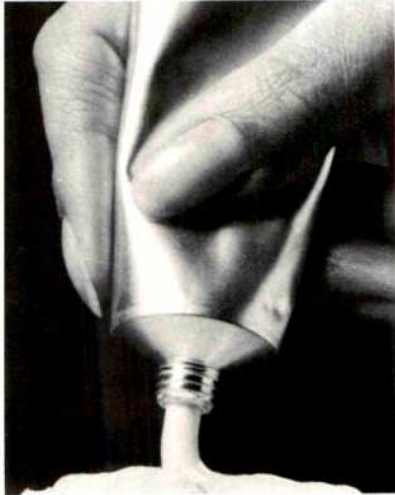
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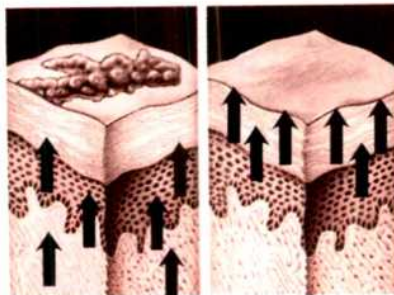


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GRISOVIN

Prescribing information

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Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.

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Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

Porphyria or severe liver disease. Griseofulvin may cause

deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions

Griseofulvin may decrease the effect of coumarin anti-coagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established.

griseofulvin

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Presentation

Tablets
125mg (bottles of 100 and 1000)
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Product Licence number

4/5060
4/5061

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Telephone 01-580 0875

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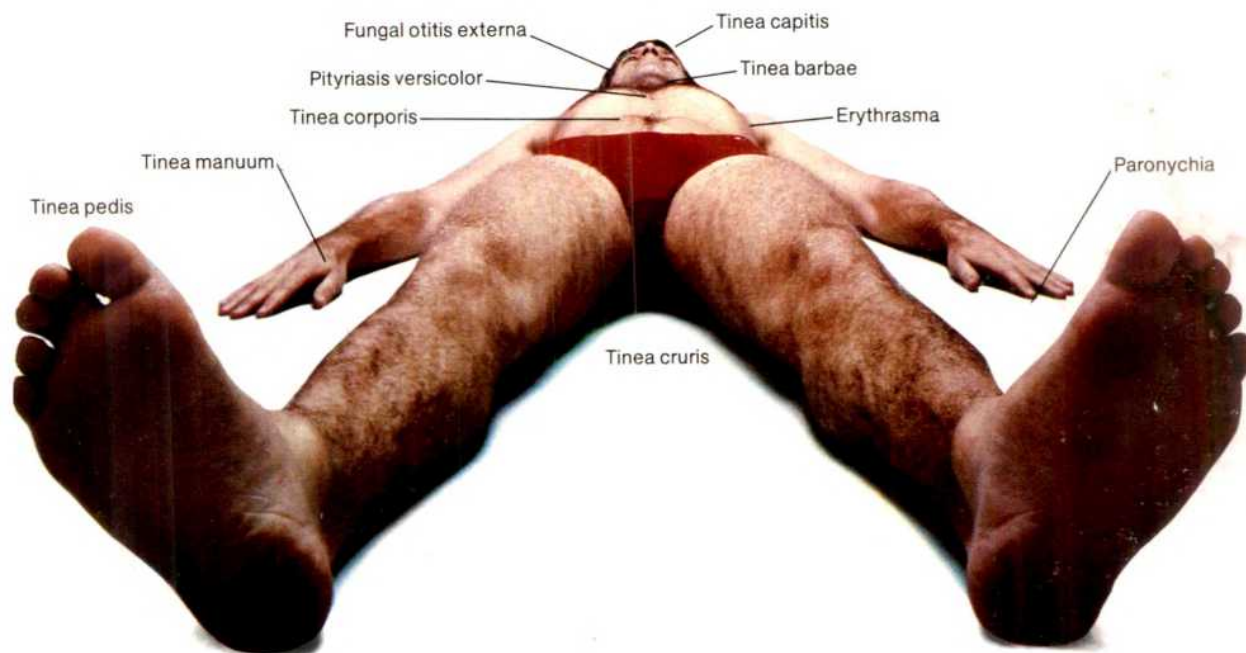
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PRESCRIBING INFORMATION

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EUMOVATE: Apply up to four times a day until improvement occurs, when the frequency may be reduced.

BETNOVATE: Apply a small quantity two or three times daily until improvement occurs. Improvement may then be maintained by applying once a day, or less often.

DERMOVATE: Apply sparingly once or twice daily until improvement occurs. Treatment should not be continued for more than four weeks without review.

Side effects

General: With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants, the nappin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.

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Precautions

Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy. With all corticosteroids, prolonged application to the face is undesirable.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

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Further information on Trimovate (trade mark) is available from:
Glaxo Laboratories Ltd, Greenford, Middlesex UB6 0HE
Product licence numbers
Ointment – PL/0004/0265 Cream – PL/0004/0266

Prescribing information

Dosage and administration

Apply to the affected area up to four times a day.

Contra-indications

Viral (e.g., herpes simplex) and dermatophyte (tinea/ringworm) diseases of the skin, and tuberculous lesions.

Precautions

In infants and children, long-term continuous topical steroid therapy should be avoided where possible, as adrenal suppression can occur even without occlusion. In infants, the napkin may act as an occlusive dressing and increase absorption. Any spread of

infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic chemotherapy. With all corticosteroids, prolonged application to the face is undesirable. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

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Debrisan a major breakthrough in wound treatment



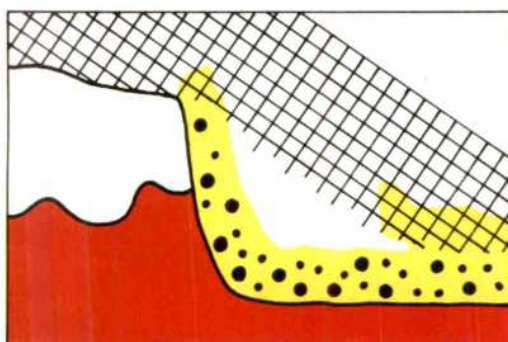
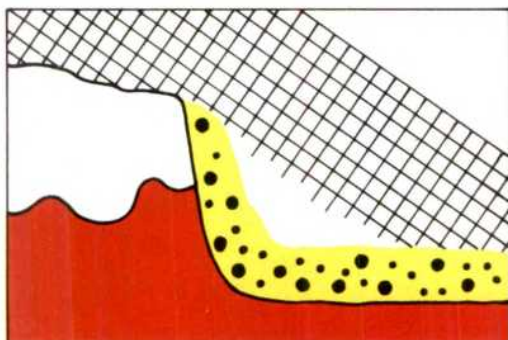
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Conventional dressing

Passive * partial *
sometimes painful



Top and bottom left

A conventional dressing may bridge the wound and hold a layer of pus against the wound surface.

Red: Oedema

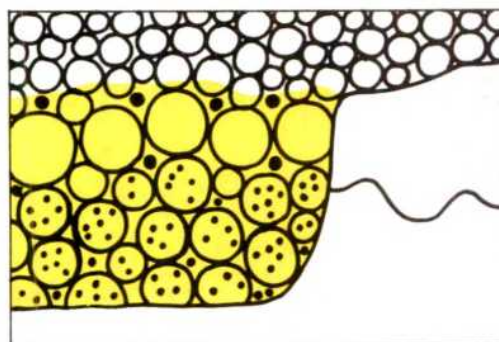
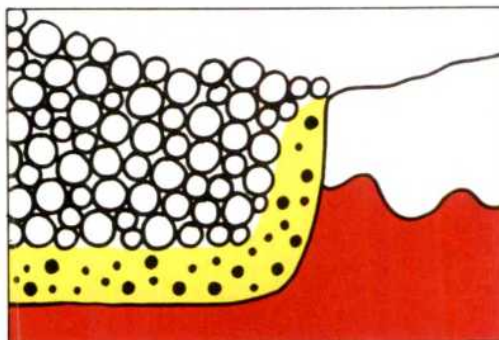
Yellow: Infected pus

Large black dots: Microorganisms and large molecules

Small black dots: Small molecules

Debrisan

active * continuous *
rapid relief of pain



Top right

Debrisan fills the wound achieving intimate contact with the wound surface.

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Wound exudate is drawn into the Debrisan layer and away from the wound surface. Cleansing is continuous if Debrisan is replaced before saturation.

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Indications

All kinds of secreting, suppurative wounds, such as post-operative wounds, leg ulcers, pressure sores, traumatic wounds, hand burns, suppurative ulcers, infected radio dermatitis, non-venereal penile ulcers.

Warning:

Debrisan spillage can render surfaces very slippery. Clear spillages promptly.

Caution:

In order to avoid cross-infection it is recommended that the contents of a castor be confined to the treatment of one patient only.

Debrisan-healing through cleansing



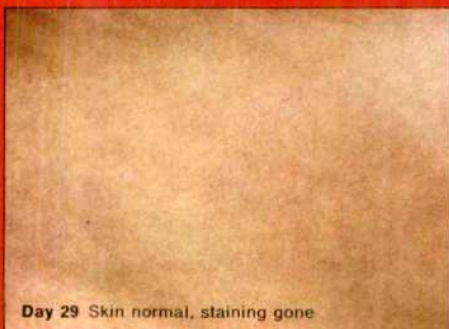
Day 1 A discrete psoriatic plaque



Day 8 After treatment with Dithrolan



Day 15 Psoriasis clear, stain remaining



Day 29 Skin normal, staining gone

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References: 1. Brit. J. Derm 93, 205, 1975. 2. Brit. J. Derm 95, 643, 1976. 3. Practitioner 219, 833, 1977.

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Natuderm is a white cream containing: **Lipid Phase:** Free Fatty Acids 14.26%, Squalene 9.4%, Squalane 1.41%, Waxes 19.97%, Sterol Esters 3.81%, Free Sterols 2.37%, Glycerides 44.27%, Phospholipids 0.48%, dl- α -tocopherol 0.0085%, Butylated Hydroxyanisole 0.0085%, Span 60 Sorbitan Monostearate 2.71%, Phenonip 1.27%. **Aqueous Phase:** Glycerol 5.63%, Polyoxyethylene Sorbitan Monostearate 1.64%, De-ionised Water 92.2%, Phenonip 0.46%. **Indications:** Conditions where skin film emulsion deficiency is a factor. **Administration:** A thin application as required. **Warnings:** For external use only. **Contra-Indications:** Nil. **Side Effects:** Hypersensitivity is a rare possibility. **Pack & Prices:** Jars of 40gms. £0.71 Jars of 450gms. £3.78 **Product Licence Number:** PL 0748/0001. *Trade Mark

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ANNOUNCEMENT



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Pellicol contains Alkyl dimethyl benzyl ammonium halide dibromide 4.0% W/W; Benzalkonium chloride 0.5% W/W. PL 0173/0016



British Patent No. 1155258

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Clinical and Laboratory Investigations

The pathogenesis of miliaria rubra

ROLE OF THE RESIDENT MICROFLORA

ERHARD HÖLZLE AND ALBERT M. KLIGMAN

Department of Dermatology, University of Munich, and Department of Dermatology, University of Pennsylvania, U.S.A.

Accepted for publication 1 February 1978

SUMMARY

Anhidrosis was induced in volunteers by covering the skin with an impermeable plastic film.

The degree of sweat suppression and miliaria after a thermal stimulus was directly proportional to the increase in the density of resident aerobic bacteria, notably cocci. No anhidrosis resulted when antibacterial substances were used to prevent the expansion of the microflora.

Histological study showed a PAS-positive diastase resistant amorphous mass deep within the acrosyringium after 2 days of occlusion, accompanied by a periductal dermal infiltration of leukocytes. After another 2 days the duct became clogged by an amalgam of degenerating leukocytes. This impaction sloughed after about 3 weeks as a result of epidermal renewal. Colonies of bacteria were never found within the ducts.

It is postulated that cocci secrete a toxin which injures luminal cells and precipitates a cast within the lumen. Infiltration by leukocytes creates an impaction which completely obstructs the passage of sweat for several weeks.

At the end of the last century, dermatological masters had realized that eccrine sweat ducts were involved in heat rash. Robinson (1884) was the first to argue that obstruction of the ducts was the primary event in the pathogenesis of miliaria crystallina. Later Pollitzer (1893) and Unna (1896) explained miliaria rubra in the same way. Yet the nature of this obstruction and the causes of its formation are unrevealed to this very day. The innocent but critical scholar who tries to read his way to an understanding of the subject will soon find that, despite important recent additions to knowledge, the pathogenesis of miliaria rubra is purely speculative.

The subject is presented in such a tidy manner in text books that one comes away with the conviction that there is little more to learn. The lack of controversy in the literature also conveys the impression that the major problems have been solved. The true situation is otherwise.

The object of this work is two-fold: First, to put the growth of knowledge about miliaria in historical perspective. Secondly, to present a series of experiments which demonstrate that the overgrowth of resident microorganisms is the central factor in causation.

Correspondence: Department of Dermatology, University of Pennsylvania, Suite 203, 3500 Market Street, Philadelphia, Pa 19104, U.S.A.

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HISTORICAL REVIEW

It was World War II which precipitated systematic investigation of miliaria. Heat rash was a common problem in troops deployed to the Pacific Theatre. Sulzberger & Emik (1946) reported that, after some weeks in the tropics, two-thirds of naval personnel suffered much discomfort and distress from this source. The rash was of minor importance in comparison to the heat intolerance syndrome which came to be known as tropical anhidrotic asthenia. The latter, with its high fever and prostration, was utterly incapacitating. Afflicted soldiers were incapable of sweating and hence lacked the mechanism for dissipating the heat load imposed by exercise in the tropics.

A series of careful investigations by Sulzberger & Zimmerman (1946) added important new insights. These men were the first to link miliaria with anhidrosis. They hypothesized that profuse sweating caused maceration of the horny layer inciting 'faulty' keratinization. This led to occlusion of the terminal portion of the sweat ducts by keratotic plugs, which blocked the delivery of sweat to the surface. How maceration caused abnormal keratinization was not explained.

At about the same time O'Brien (1947) was conducting his pioneering investigations in Australia. He too realized there had to be an obstruction but he was more vague regarding its exact nature. He used the term 'poral closure' to describe a functional closing of a 'keratin ring' around the sweat pore. The dammed back sweat then burst through the epidermal segment of the ducts, producing the inflammatory vesiculo-papules of miliaria rubra. Repeated bouts of rupture eventuated in parakeratotic horny plugs which obstructed the ducts even more deeply, setting the stage for miliaria profunda which resulted from rupture of the ducts within the dermis. At first, O'Brien attributed poral closure to lipid depletion of the 'keratin ring'. He formulated this theory after observing that liberal application of lanolin to anhidrotic skin would almost immediately restore sweating. Moreover, frequent washing with soap or repeated applications of lipid solvents induced miliaria rubra in soldier volunteers. Biopsies showed the familiar parakeratotic plugs.

Later, O'Brien (1950) viewed lipid depletion as only one of a number of predisposing factors. Subsequent studies led him to postulate a key role for *Staphylococcus aureus*. He came to regard miliaria rubra as a staphylococcal infection of the sweat ducts. He published photos showing crowds of cocci colonizing the distal portion of the ducts. Moreover, he was able to create miliaria rubra in volunteers by fastening to the skin agar plate cultures of *S. aureus*. This procedure also created lesions of bullous impetigo which he regarded as related to miliaria rubra.

These developments were soon followed by the classic studies of Shelley & Horvath (1950a, b). They used a variety of experimental manoeuvres to produce miliaria crystallina and miliaria rubra: these included continuous wet dressings, iontophoretic current, occlusive adhesive tape and applications of aluminum chloride. They established that a variety of superficial injuries could cause parakeratotic and hyperkeratotic plugs to form in the distal portion of the sweat ducts.

These findings neatly interdigitated with the observations of Sulzberger and of O'Brien and provided the foundation for current teaching of the origin of miliarial syndromes. Horny plugs are central in this version of pathogenesis. They prevent the delivery of sweat to the surface and their depth determines the level at which sweat escapes into the tissue. Miliaria crystallina (intra-corneal vesicles) develops when the plug is situated in the horny layer, miliaria rubra when it is further down in the epidermis and finally miliaria profunda with dermal blockage. This picture was fated to be disturbed by new findings. Hambrick & Blank (1956) microscopically examined whole mounts of miliaria crystallina lesion. They could not find plugs of any kind. Dobson, Lobitz & Hanover (1957) reopened the field to critical thinking by following the very early histological events after different physical and chemical injuries. The skin quickly became anhidrotic after freezing, but the much described horny plugs were nowhere in evidence. The injury was severe enough to cause degeneration of the

epidermal sweat duct unit. It was only after repair of this damage that parakeratotic plugs formed. They postulated that an accumulation of PAS-positive diastase resistant material in the ducts was responsible for obstructing the flow of sweat to the surface. They clearly perceived that anhidrosis developed in the absence of horny plugs and that these come later as a manifestation of the repair process. The idea of some chemical substance blocking the ducts received support from the observations of Cormia & Kuykendall (1954). They wished to account for the fact that sweating was absent in lesions of chronic dermatoses like psoriasis. They too found amorphous masses of PAS-positive material within the ducts.

Papa & Kligman (1966) emphasized that earlier researchers had concentrated their efforts on the miliarial lesions, overlooking the antecedent stage of anhidrosis when horny obstructions were not demonstrable. By applying toxic chemicals, such as formalin and trichloroacetic acid, an anhidrotic state was quickly established in the absence of horny plugs. The terminal ducts contained an amorphous material. When this was removed by stripping with scotch tape, sweating was immediately restored. They too observed PAS-positive casts in the ducts but these formed only after an obstruction had been established. These casts were not constantly present and were viewed as products which accumulated behind the block rather than constituting the primary obstruction as Dobson *et al.* (1957) had thought.

Once more the tragedy of war, this time the Vietnam experience, brought to the fore the importance of miliaria rubra in limiting the effectiveness of fighting troops. The basic question remained: How does profuse sweating induce miliaria in persons who are newcomers to tropical areas? In a return engagement with the problem, Sulzberger, Griffin & Wiley (1967), at the Letterman Army Institute, developed an experimental model which perfectly mimicked the natural disease. This provided an unprecedented opportunity for examining the pathogenesis of miliaria. By wrapping up the torso with tightly fitting impermeable plastic film (Saran Wrap) for 2-4 days, they could regularly provoke miliaria rubra in heat-stimulated volunteers.

With this model, Stillman, Hindson & Maibach (1971) studied the effect of various pretreatments, hoping to find an effective means of prophylaxis. They simultaneously estimated the degree of anhidrosis and miliaria and found a fairly good correlation between the two. They were able to moderate sweat retention by a variety of applications including lanolin, acid buffers, a topical corticosteroid, its cream base and two local antibiotics (neomycin and chloramphenicol). The diverse nature of these pre-treatments did not indicate a common mode of action. The authors could offer no new thoughts regarding the aetiology of miliaria rubra.

Henning, Griffin & Maibach (1972) used the same model to assess the role of bacteria. Marples & Kligman (1965) had shown that occlusive dressings for 24 h brought about a great expansion of the microflora, from hundreds of organisms per cm² to hundreds of thousands. Henning *et al.* (1972) semi-quantitatively estimated the number of resident micro-organisms before and after occlusive wrappings for 48 h. A strict correlation between bacterial density and the degree of anhidrosis could not be demonstrated. The authors did not postulate a bacterial aetiology for miliaria rubra.

Meanwhile others were finding that occlusion for a few days would regularly induce anhidrosis. In an examination of tapes of differing occlusivity both Orentreich, Berger & Auerbach (1966) and Gordon & Maibach (1969) found that only those tapes which were completely impervious to water could induce a complete anhidrosis. The most striking finding in these studies was the long duration of reduced sweating. It took 2 or 3 weeks for sweat patterns to be fully restored. Scotch tape stripping did not relieve the anhidrosis, a situation quite different from the 'high level blockade' of Papa & Kligman's (1966) chemically induced anhidrosis.

In still another study, Sulzberger & Harris (1972) wished to find out what the earliest anatomical changes were in their occlusion model. They carefully examined histological sections after various

periods of occlusion. They were disappointed in not being able to identify any change that would explain the lack of sweating or how a heat stimulus precipitated miliaria. There was no visible obstruction, horny or otherwise. Their own statement best describes how matters lay at that time: 'The antecedent to the histological changes of sweat duct leakage or rupture and the gross occurrence of miliaria still eludes us.'

We may state the central problem as follows: When the skin of normal persons is completely occluded for a few days a durable anhidrosis results. The question is: What is the nature of the obstruction that prevents delivery of sweat to the surface? There is no disagreement that it is the extravasation of sweat into the skin which causes miliarial syndromes. It is the first process, the stage of anhidrosis, that is still mystifying.

MATERIALS AND METHODS

The studies were carried out during the winter months to exclude such variables as profuse sweating and sun exposure. Fifty-five (55) healthy young, black and white adults of both sexes served as paid volunteers. Each study utilized a group of 5-8 persons. The volar forearms or the lower back was the test site. Non-medicated soaps, but nothing else, were permitted.

Occlusion procedures

Squares of skin 5×5 cm were covered by a single layer of vinylidene polymer film (Saran Wrap, Dow Chemical Co., Indianapolis, Ind.) cut between sheets of paper. The plastic film was sealed to the skin under overlapping strips of impermeable tape (Blenderm, 3M, St Paul, Minn.). This, in turn, was further fixed by an elastic adhesive bandage (Elastoplast, Beiersdorf, Inc., S. Norwalk, Conn.) completely encircling the forearm. This arrangement assured uniform, firm contact of the film with the skin. To achieve a similar pressure effect on the back a sheet of foam rubber, $5 \times 5 \times 0.6$ cm, was sandwiched between the impermeable tape and the elastic bandage. Two test sites could be prepared on one forearm or on one side of the back.

Evaluation of sweat suppression

Sweating was induced by heat stimulation in an environmental chamber at 55°C and 60% R.H. After the onset of profuse sweating, usually about 10 min, sweating was assessed by a modification of Sarkany & Gaylarde's (1968) imprint method. A quick setting, hydrophobic silicone polymer (Syringe Elasticon, Kerr, Romulus, Mich.) was layered onto the skin trapping bubbles of sweat for a 1 min period. This provided a permanent record. A semi-quantitative estimate of sweat suppression was made by comparing the number of droplets in treated and control skin. The degree of hypohidrosis was expressed in a five point scale: 0, 25, 50, 75 and 100% sweat inhibition. Fig. 1 illustrates that it is fairly easy to assess the degree of sweat suppression provided that one assays no greater accuracy than the nearest 25%. An excellent correlation was found between this global assessment and actual counts of sweat droplets determined by projecting the imprints on a screen.

Microbiological analysis

The density of aerobes was determined by the detergent-scrub method of Williamson & Kligman (1965). Two 1 min scrubs were pooled using 1 ml of 0.1% tyloxapol (Triton X-100, Rohm & Haas, Philadelphia, Pa.) in a glass cup having an area of 3.8 cm^2 . Tenfold serial dilutions were made to 10^{-4} . Then 0.025 ml of each dilution was drop-plated from a 0.2 ml pipette onto the following media:

- (1) Trypticase Soy Agar, a general medium.

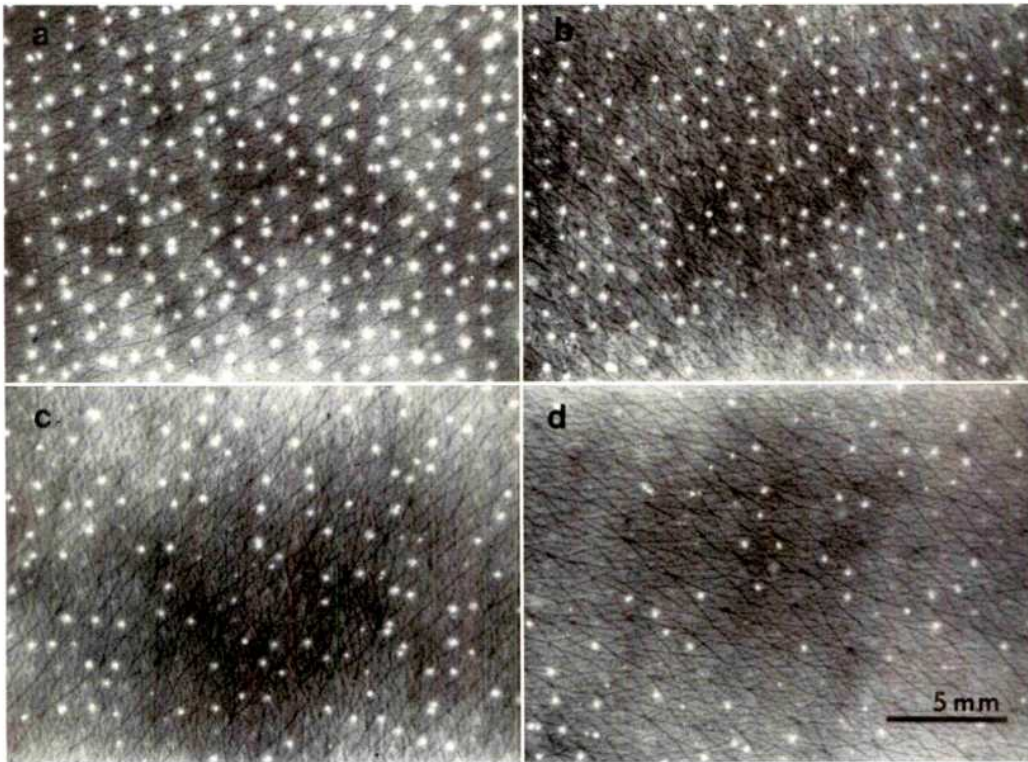


FIGURE 1. Silicone imprints from the back viewed in transmitted light. (a) Normal sweat pattern; (b) 25%; (c) 50%; and (d) 75% sweat suppression.

- (2) Trypticase Soy Agar with added Lecithin and Polysorbate 80 to enhance the growth of lipophilic diphtheroids.
- (3) Littman-Oxgall agar for the isolation of yeasts.
- (4) McConkey's agar for isolation of Gram negative bacteria.

STUDIES

Experiment 1: effect of hydration

Since the days of Pollitzer (1893) swelling of the horny layer by water has been linked to closure of the ducts. Sulzberger & Zimmerman (1946) implicated the imbibition of sweat as the factor leading to faulty keratinization, and Shelley & Horvath (1950 b) induced miliaria rubra by wet dressings.

Randall & Peiss (1957) observed that after immersion of the palms in water for 60 min, sweating ceased and was not restored until about an hour later, when the surface had again become dry. Immersion in a hypertonic solution of 20% NaCl had no effect because swelling of the horny layer did not occur. It could be argued that persistent anhidrosis is simply a result of intense hydration of the horny layer, leading to 'poral closure'.

Method

Wide rimmed plastic chambers 2.5 cm in diameter, containing 5 ml sterile, deionized water were fixed

to the volar forearms of 5 subjects for 1, 3, 6, 12, 18 and 24 h. The cups were sealed to the skin by means of adhesive (30-1289, National Starch and Chemical Corporation, Bridgewater, N.J.) and further secured by encircling the limb with adhesive elastic tape. Sweating was evaluated immediately upon removal of the chambers. In another 5 subjects, the water chambers were applied for 24 h and sweating assessed immediately, 15, 30, 45, 75 and 180 min after removal.

Results

One hour of hydration had no effect. By 3 h there was a 25% suppression. This gradually increased to become complete or nearly so in 24 h.

In the second group, 24 h of hydration also produced virtually complete anhidrosis. After exposing the skin to the air, sweat suppression had already begun to wane within 10 min. Recovery was complete after 90 min (Fig. 2).

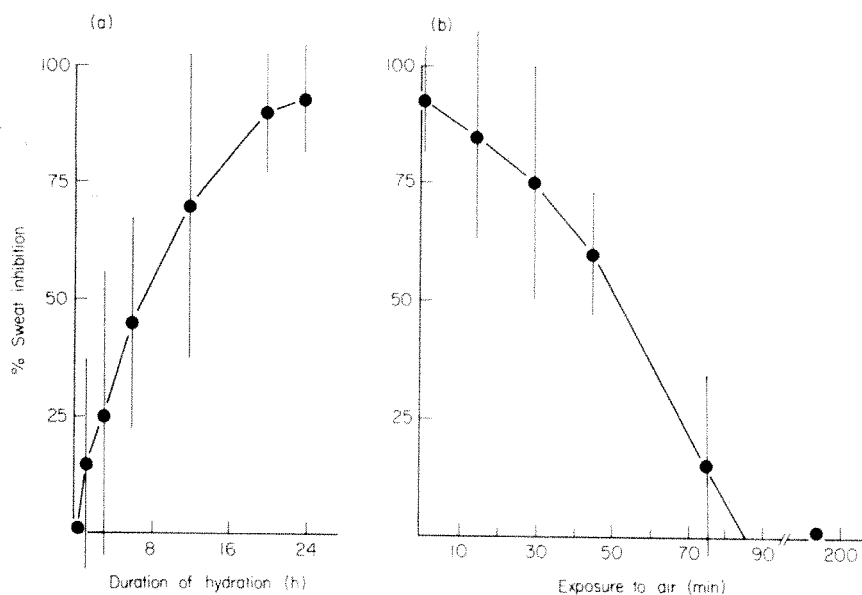


FIGURE 2. (a) Increase of hypohidrosis during hydration. (b) Decay of sweat inhibition after hydration for 24 h. Means and s.d.

Comment

The cessation of sweating is obviously much more rapid on the palms and soles where the horny layer is very thick. On the glabrous skin, it takes about 24 h to achieve complete anhidrosis. The suppression of sweating is short-lived with 50% recovery in about 50 min and complete restoration in about 1.5 h. Preliminary experiments on the back revealed that hydration for 24 h also produced a marked hypohidrosis, but to a lesser degree and for a shorter period than on the forearms.

Papa & Kligman (1966) also observed that hydrating the skin with wet patches for 24 h would produce a temporary anhidrosis. Sweating could be instantly restored by Scotch tape stripping, immediately after removing the dressings, indicating a high level blockade. The obliteration of the pores on the palms has been visualized using plastic replicas by Sarkany, Shuster & Stammers (1965). The slit-like openings of the ducts on the glabrous skin cannot be seen in silicone replicas.

Since the horny layer is capable of taking up 500-600% of its dry weight after immersion in water

for 24 h, swelling of the horny cup through which the terminal eccrine duct winds furnishes an adequate explanation for poral closure. It is important to appreciate the existence of temporary sweat suppression from hydration. By waiting 2–3 h after removing the occlusive dressing, the hydration effect can be ignored. This rule has been observed in all the studies which follow.

Experiment 2: dynamics of anhidrosis and miliaria

Occlusion of the skin by impermeable plastic dressings for several days produces an anhidrosis, or more properly, a marked hypohidrosis, which lasts for at least 2 weeks. During the anhidrotic state a heat stress may precipitate a miliarial eruption. This takes different forms depending on the level of the obstruction. Questions remain concerning the dynamic relationships between miliaria crystallina, miliaria rubra and miliaria profunda. Shelley & Horvath (1950 a, b), for instance, produced miliaria crystallina and miliaria rubra by similar experimental manoeuvres; there is much uncertainty concerning the determining factors.

Method

Anhidrosis was established by occluding forearm or back skin for 4 days with plastic film. At various times thereafter, sweating was induced in the environmental chamber noting the type of eruption produced and its intensity. The degree of sweat inhibition was monitored as well.

Results

After removing the dressings, a substantial anhidrosis was present in every subject ($80\% \pm 20$). Sweat suppression continued at that level or even increased somewhat during the first week. Thereafter, more and more ducts became capable of delivering sweat. By 14 days suppression averaged 25% (Fig. 3). In a few persons a 50% inhibition was detectable even after 3 weeks.

On the back almost every subject exhibited miliaria rubra, the severity of which was directly proportional to the degree of sweat inhibition. Miliaria was also elicited on the forearm but there it occurred less often and was considerably less intense, showing a few scattered tiny papules.

As a rule, mild miliaria rubra was already evident at the time the patches were removed, though forced sweating greatly intensified the reaction. However, after several days, the eruption acquired

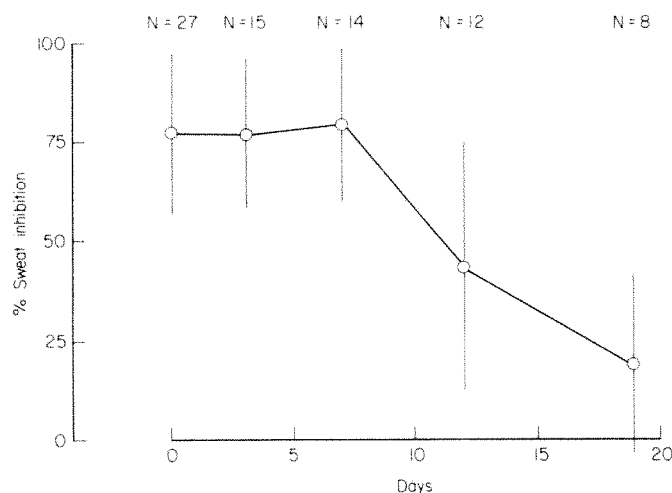


FIGURE 3. Duration of hypohidrosis after 96 h of occlusion. Means and s.d.

different characteristics. The papules were generally less red, often larger and weal-like, features resembling miliaria profunda. Yet, unequivocal miliaria profunda was not common, occurring in only 12% of the cases after a week. It generally could not be provoked after about 2 weeks. Shortly before the restoration of the normal sweat pattern, the test sites exhibited moderate scaling. At that stage, thermal stimulation precipitated miliaria crystallina in subjects who had previously experienced miliaria rubra. Usually careful examination under the stereomicroscope was necessary to visualize the microvesicles. In some persons, the classical dew-drop vesicles appeared.

Comment

These observations can be found in the works of others but this is perhaps the first time that serial observations have been made from the onset of anhidrosis. Since the turnover of the stratum corneum is about 2 weeks and it takes about 3 weeks for complete restoration of sweating, it seems likely that the obstruction extends into the epidermal portion of the sweat duct. The block is abolished when the tissue is renewed from below. Griffin, Maibach & Sulzberger (1967) have also suggested that the duration of anhidrosis is related to renewal of the epidermis. They too noted a close correlation between the degree of anhidrosis and the intensity of miliaria. However, it is not always appreciated that it is not the miliaria which causes the anhidrosis but the other way around. The repair of severe epidermal damage after miliaria rubra produces parakeratotic plugs. These of course sustain the anhidrosis and deepen the obstruction. In this way, repeated bouts of miliaria rubra give rise to miliaria profunda. Miliaria crystallina is the final expression in this sequence. When the obstruction has risen to the level of the horny layer, rupture at this superficial level produces intracorneal vesicles.

Miliaria crystallina, rubra and profunda are related aspects of the same process. Injury, repair and epidermal turnover are interlocked in creating the observed sequence.

Experiment 3: the role of the microflora

The idea that micro-organisms might play a role in miliaria dates back to the speculations of Unna (1896) and Acton (1926). O'Brien (1950) held that *S. aureus* colonized the ducts and was responsible for producing miliaria as well as bullous impetigo. Lyons, Levine & Auld (1962) sampled the microflora by means of a semi-quantitative touch plate method and found the density of resident organisms to be threefold higher in miliaria subjects. Pathogenic staphylococci were most often recovered. Henning *et al.* (1972) assessed the role of bacteria in miliaria but their findings did not support the concept of bacterial causation. Indirect evidence of the participation of surface organisms after occlusion was furnished by Singh (1973) who was able to prevent sweat suppression by topical applications of antibiotics. In another experiment he observed some correlation between the degree of anhidrosis and microbial density under occlusive dressings.

Method

Sweat suppression was induced in 27 subjects by occlusion with plastic film for 4 days. The density of aerobes was quantitatively determined. In a special group of six subjects, occlusion was maintained under separate patches for 1, 2, 3 and 4 days according to a plan by which all the dressings could be removed at one time.

Results

The composition of the microflora on volar forearms or the back after occlusion for 96 h is shown in Table 1. The dominant organisms in nearly every instance were coagulase negative micrococci with a geometric mean count of $6.44 \times 10^6/\text{cm}^2$. Aerobic diphtheroids were present in about 50% of the cases and in lesser numbers. Yeasts and Gram negatives were not frequent. Micrococci were absent

TABLE 1. Aerobic microflora after occlusion for 96 h (N = 27)

	Incidence (%)	Geometric mean of counts/cm ² when present (log ₁₀)
Coagulase negative micrococci	96.2	6.44 ± 0.48
Aerobic diphtheroids	48.1	5.88 ± 0.51
Gram negative bacteria	3.7	3.00
Yeasts	11.1	4.69 ± 0.68

in only one instance in which yeasts comprised the entire flora. There was no sweat suppression in this case. In all other instances substantial anhidrosis was induced by occlusion ($80\% \pm 20$). After only 24 h of occlusion, the mean density of micrococci exceeded $1 \times 10^5/\text{cm}^2$. However, sweat suppression was only slight at that time. The counts were a little higher after 2 days of occlusion and this was accompanied by a moderate increase in sweat suppression. Yet it was not until after 3 days that 50% inhibition was obtained. The degree of anhidrosis swung sharply upwards with another 24 h of occlusion (Fig. 4). It must be remembered that these data do not refer to the temporary anhidrosis of hydration. Sweating was always assessed after the horny layer had been allowed to dry out.

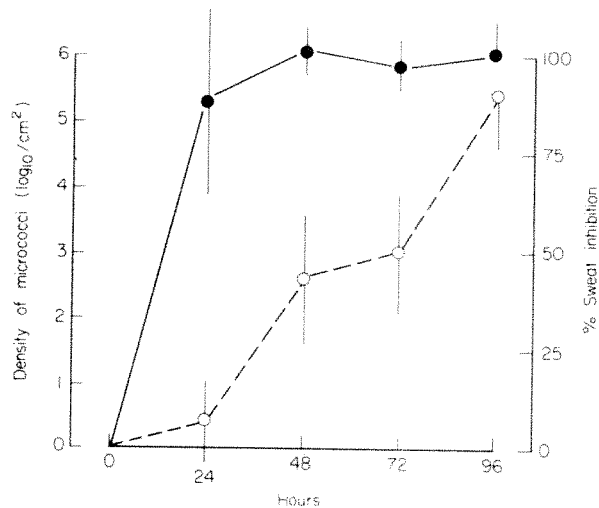


FIGURE 4. Density of micrococci (●) and development of sweat inhibition (○) under plastic film occlusion. Means and s.d.

Comment

These data strongly suggest a correlation between the development of anhidrosis and the growth of micrococci; the latter are the dominant members of the normal cutaneous microflora in virtually all areas of the body. Anhidrosis was not influenced by the presence or absence of diphtheroids. Yeasts and Gram negatives need not be seriously considered. These were not present in most cases and were generally sparse. They did not, however, interfere with the development of anhidrosis. In

other studies we have sometimes observed pure cultures of yeast on sites treated with antibacterial antibiotics. In none of these instances was there any sign of sweat suppression.

It is noteworthy that the anhidrosis lags appreciably behind the expansion of the micrococci; the latter are nearly at peak densities by 24 h. However, it takes about 4 days for maximum sweat suppression to be attained.

Experiment 4: effect of suppressing the microflora

One can find, in the works of others, observations which support a role for bacteria in the aetiology of miliaria. However, substantive proof has not been brought forward. In the studies of Stillman *et al.* (1971), topical antibiotics were no more effective in preventing miliaria than lanolin or corticosteroids. Singh (1973) felt that pretreatment with antimicrobial substances could prevent anhidrosis, but in this study no bacteriological data were presented and the degree to which bacterial growth was inhibited is not known. Proof of the micrococcal thesis requires the demonstration that occlusion will not induce anhidrosis if bacteria are prevented from multiplying.

Method

0.1 ml solutions of various antibacterial substances were spread over 5×5 cm squares by means of a glass applicator. After drying, the sites were occluded in the usual way.

The test agents were:

Povidone iodine (Betadine, Perdue Frederick Company, Norwalk, Conn.).

0.25% Mg-pyrithione (H_2O) (Mg-Omadine, Olin Mathieson Chemical Corporation, New Haven, Conn.).

1% Hexachlorophene (ethanol).

1% Zinc bacitracin (suspended in H_2O).

1% Chloramphenicol (50% ethanol).

1% Erythromycin (50% ethanol).

70% Ethanol.

The control was physiological saline solution. With 70% ethanol the skin was degermed as follows: A gauze pad soaked in 70% ethanol was applied for 2 min; then, with a similar fresh pad the surface was gently rubbed for 15 s. This sequence was repeated twice. The test substances were applied once daily for 4 days (four subsequent occlusion periods of 24 h each). Immediately after removing the last patch, the sites were sampled bacteriologically; 3 h later sweat inhibition was evaluated.

Results

At the end of 96 h, the geometric mean count for the control sites was of the expected magnitude, about $1 \times 10^6/cm^2$. With 3 agents, magnesium pyrithione, hexachlorophene and zinc bacitracin, there was no expansion of the flora, the counts being even lower than normal. There was no sweat suppression at these bacteria-poor sites. Ethanol swabbing also held the organisms in check and this too was associated with normal sweating. Erythromycin only moderately inhibited bacterial growth. This was true for chloramphenicol also but to a lesser extent. Anhidrosis was only partial with these two antibiotics. In both instances, the explanation for the modest suppression of the microflora was the emergence of resistant strains.

When the percentage of sweat suppression was plotted against the density of micrococci (leaving out counts below normal), a linear relationship was found ($r = 0.94$, $P < 0.001$); the fewer the organisms the less the sweat suppression. Anhidrosis was virtually complete when the counts reached $1 \times 10^6/cm^2$ (Fig. 5).

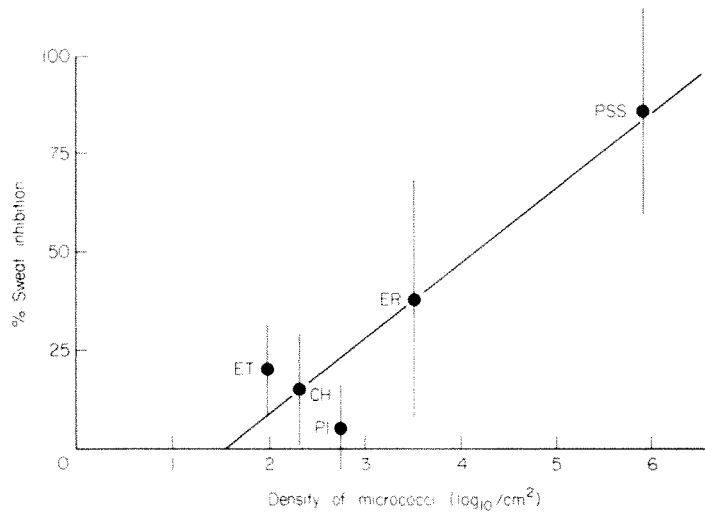


FIGURE 5. Influence of antibacterial agents on density of micrococci and sweat suppression. Hypohidrosis parallels the growth of organisms. Means and s.d.; PSS = 0.9% NaCl, PI = povidone iodine, ET = 70% ethanol, CH = 1% chloramphenicol, ER = 1% erythromycin. $y = 0.8x - 30$.

Comment

The parallel relationship between the quantity of aerobes, notably micrococci, and the degree of sweat suppression strongly supports a causal role for these organisms. In turn, the frequency of miliaria increases proportionally with the density of resident organisms.

Our results with chloramphenicol and erythromycin are rather similar to those of Stillman *et al.* (1971). Sweat suppression and curtailment of miliaria were only obtained to a modest degree. This can be attributed to the development of resistant strains during the 4-day exposure period. With orally administered tetracycline, Marples & Kligman (1971) noted that resident organisms uniformly became resistant within a few days.

The alert reader may have wondered why data are not given for neomycin, the most widely used topical antibiotic. As a matter of fact, we prevented the expansion of the microflora and miliaria with both neomycin sulphate and polymyxin B sulphate. In several subjects, however, a paradoxical result was obtained. Anhidrosis developed even though the bacterial density was very low. This puzzle was solved by further studies which showed that neomycin and polymyxin acted directly as antiperspirants. Topical application of 5% solutions under occlusion for 72 h resulted in substantial sweat inhibition. To our astonishment intradermal injection of 0.5% polymyxin B rendered the treated skin anhidrotic for almost 3 weeks; neomycin was ineffective when injected.

We postulate that these antibiotics have anticholinergic effects. Support for this view is provided by the observations of Timmerman, Long & Pittinger (1959), who found that neomycin and polymyxin B were capable of blocking the neuromuscular junction. Thus, these particular antibiotics comprise special cases.

Experiment 5: effects of various occlusive systems

Orentreich *et al.* (1966) and Gordon & Maibach (1969) found that the degree of hypohidrosis induced by tapes was related directly to their occlusiveness. Permeable tapes were ineffective. Likewise,

Marples & Kligman (1969) found that occlusive tapes greatly stimulated bacterial growth while porous ones were associated with low densities of resident aerobes. In this experiment, dressings of differing occlusivity were used to attain different levels of bacterial growth furnishing a means to examine in more detail the quantitative relationship between bacterial density and the degree of sweat suppression.

Method

Forearm skin was covered for 96 h with the following materials:

- (1) Zonas (Johnson & Johnson, New Brunswick, N.J.)—a semi-occlusive cloth tape, with an adhesive backing containing ZnO.
- (2) Dermicel (Johnson & Johnson, New Brunswick, N.J.)—a porous cloth tape, non-occlusive.
- (3) Blenderm—an impermeable plastic tape, highly occlusive.
- (4) Plastic cups, 25 mm in diameter, 5 mm in height.

Plastic film (Saran Wrap) served as the control. The flora was sampled immediately after removing the dressings and sweating was evaluated 3 h later.

Results

Blenderm and Saran Wrap yielded similar results. Both are completely occlusive and create conditions for resident bacteria to attain peak densities. Anhidrosis was nearly complete with both. By contrast, Dermicel, a porous cloth tape, did not hydrate the horny layer. Neither sweating nor the bacterial count was importantly changed. Zonas provided still another type of result. The adhesive mass of this tape is antibacterial probably due to the presence of zinc compounds. Inhibition of bacterial growth was complete and accordingly there was no sweat suppression. The plastic cup allowed for a variable bacterial expansion; the degree of hypohidrosis in every instance paralleled the density of organisms. No sweat suppression occurred with counts only slightly above normal. Fig. 6 shows a plot of the density of micrococci against percentage sweat inhibition. Again, there was an excellent correlation ($r = 0.95$, $P < 0.001$).

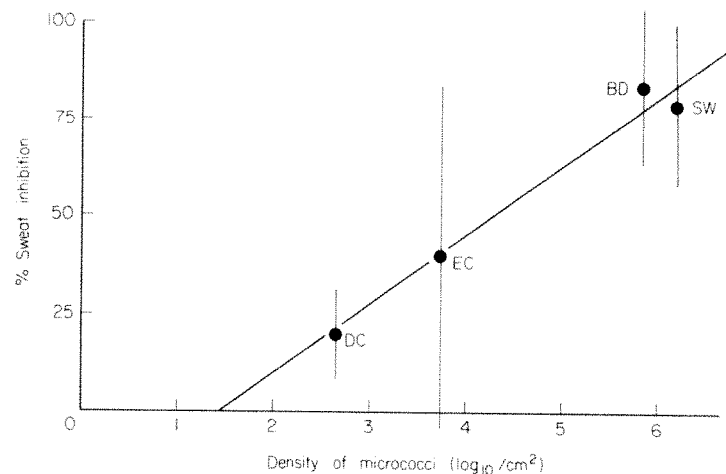


FIGURE 6. Influence of dressings of differing occlusivity on bacterial density and sweat suppression. Hypohidrosis increases linearly with the growth of organisms. Means and s.d.; SW = Saran Wrap. BD = Blenderm, EC = empty cup, DC = Dermicel. $y = 0.7x - 25$.

Comment

Our findings generally agree with the results of Gordon & Maibach (1969) and Orentreich *et al.* (1966). Willis (1973), too, found that even empty cups sealed to the skin caused appreciable sweat suppression. Gordon & Maibach's (1969) observation that an occlusive tape with a Zn-oxide backing (Hy-tape) caused the most pronounced anhidrosis is in conflict with our findings. In our hands, a similar tape suppressed bacterial growth; the lack of anhidrosis is, therefore, consistent with the hypothesis that bacteria play a decisive aetiological role.

Experiment 6: superhydration

Water is not exactly harmless to skin. In order to study its baleful effects, Willis (1973) fixed water-containing cups to the skin for 5 days and more. A mild dermatitis regularly developed in these superhydrated sites. However, the sites sweated freely, an entirely unexpected result in view of the great hydration of the horny layer. We repeated Willis' study with the addition of quantitative bacteriological analysis.

Method

Water filled plastic-cups (see experiment 1) were fixed to the forearm for 96 h. The detergent-scrub method was used to sample the surface growth of aerobes immediately upon removing the cups. The quantity of organisms in the water itself was also determined. Sweating was assessed 3 h later.

Results

Like Willis (1973), we found little or no inhibition of sweating. The mean surface density of coagulase negative micrococci was only $8.8 \times 10^3/\text{cm}^2$. Insignificant quantities of cocci, sometimes none at all, were recovered from the water itself. Occasionally Gram negatives reached surface densities of $1 \times 10^5/\text{cm}^2$; these sites also sweated normally.

Comment

The bacteriological findings were rather surprising. Apparently, water-filled cups establish an environment unsuitable for the growth of the resident aerobes. The surface density of these organisms was quite low and even lower counts were found in the free fluid. Perhaps conditions are too anaerobic in the cups. The absence of anhidrosis is, thus, not so surprising after all. This study further supports the concept that resident organisms play a central role in anhidrosis.

Histological studies

All are agreed that occlusion with plastic film establishes a durable anhidrosis which cannot be relieved by Scotch-tape stripping. Therefore, the block is deeper than the horny layer. What is the nature of the obstruction? To study this question, biopsies were taken at various times after the impermeable film was applied.

Method

Excisional biopsies were removed from anhidrotic skin after 15 min of thermal stimulation. Twelve specimens were removed at 2 and 4 days of Saran Wrap occlusion and again 7 days later. After formalin fixation, 10 μm sections were cut serially and stained with H & E and with PAS. In addition, to prevent mechanical dislodgement of the bacteria during processing, shave biopsies were taken after 96 h of occlusion and embedded in 8% hard agar before fixation in formalin. These sections were then stained for bacteria by the method of Brown & Brenn (1931).

Results

In agar embedded specimens, sparse numbers of cocci were inconsistently demonstrable in the sweat pore orifices. At best, a few organisms were sometimes present in the most distal portion. Bacteria were never found within the epidermal segment. Nothing resembling bacterial plugs or colonies was ever observed.

It was possible to view the earliest histological changes in specimens from those few subjects in whom anhidrosis and miliaria were already present after only 48 h of occlusion. There was a marked periductal accumulation of inflammatory cells consisting predominantly of mononuclears and some neutrophils at the level of the dermo-epidermal junction. A few leukocytes had migrated into the epidermis but none had infiltrated the ducts themselves. An eosinophilic amorphous cast was present within the deepest epidermal portion of the ducts. This was PAS-positive and diastase resistant (Fig. 7). After 4 days of occlusion, a dense periductal infiltration of mononuclears extended down-



FIGURE 7. Forty-eight hours of occlusion. An amorphous cast has formed in the epidermal duct. There is a periductal infiltrate in the upper dermis (H & E).

wards along the ducts to the mid-dermis. Many lymphocytes and a few neutrophils had by now invaded the intra-epidermal portion of the ducts, including the lower stratum corneum. The casts occupied the entire epidermal duct including the intracorneal segment. The casts, together with inflammatory cells, formed a dense conglomerate (Figs 8 and 9). One week after occlusion for 96 h, a

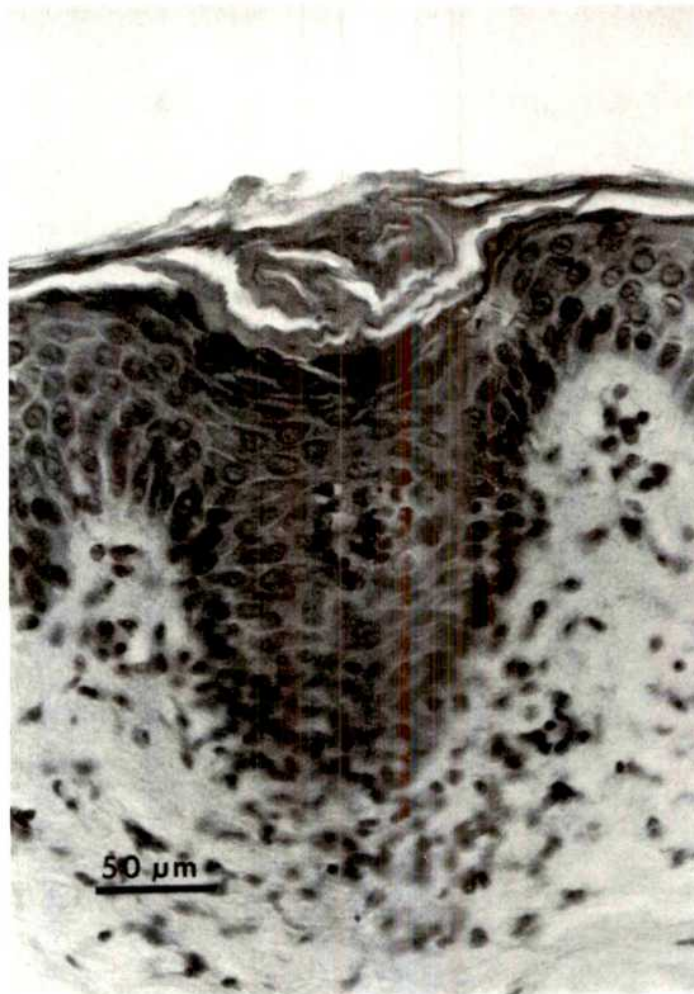


FIGURE 8. Ninety-six hours of occlusion. Degenerating leukocytes have formed a conglomerate throughout the intraepidermal portion of the duct (H & E).

periductal infiltrate was still present but was less prominent than at 4 days. The notable feature at this stage was the jamming of the ducts with a mass of cell debris, especially pyknotic nuclei from degenerating leukocytes. This solid impaction extended from the upper epidermis through the entire stratum corneum (Fig. 10). The lower epidermal and dermal part of the duct now appeared to be dilated.

Although miliaria was clinically recognizable, there were no discontinuities in the ductal lining that could be called ruptures. Some spongiotic changes were evident; however, the lining cells themselves were curiously unaffected during the entire process.



FIGURE 9. Ninety-six hours of occlusion. A dense impaction of degenerating leukocytes is present in the duct. There is a considerable infiltrate of mononuclears periductally in the dermis (H & E).

Comment

It can be categorically stated that bacteria themselves do not obstruct the flow of sweat by massing in the sweat ducts. Careful searching with special stains showed only a few cocci in the orifices, inconsistently, if that. Nothing resembling O'Brien's bacterial 'plugs' was ever seen. In a few cases of established anhidrosis, we examined the distribution of bacteria on the surface by pressing a strip of double faced Scotch tape to the skin. The adhesive was then melted by heat from an infra-red lamp. Thirty minutes later, the tape was peeled off, fixed to a glass slide with the skin surface side up and stained with Giemsa. Such 'skin surface biopsies' from sites which had been occluded for 4 days often showed clusters of cocci around sweat pores as well as around the follicular orifices with very few organisms in between. This localization of resident organisms around sweat pores was also described by Noble (1975). It should be emphasized that the organisms were visualized not in the pore itself but in its immediate vicinity.



FIGURE 10. One week after occlusion for 96 h. The agglomerate now occupies the intracorneal portion of the duct. The duct within the epidermis is normal but dilated (H & E).

We believe we have at last demonstrated the obstruction in miliaria rubra. At the earliest stage, after 2 days of occlusion, a cast of PAS-positive material was found deep within the acrosyringium just above the level where the duct enters the epidermis. Since sweating was already suppressed at this early stage, we consider that the PAS-positive cast impedes the flow of sweat. How the cast forms is a matter for speculation. We conceive that the bacteria concentrated around the sweat pores secrete a 'toxin' which slowly diffuses down the ducts. The latter are open channels for water soluble substances. When after a few days the concentration of this 'toxin' becomes high enough, a complex forms with cuticular material of the luminal cells, perhaps damaging cell membranes, which then become more permeable. Materials leak out into the lumen and precipitate as an amorphous cast. It should be noted that the cast forms deep within the acrosyringium at the level where the ductal cells have not yet begun to keratinize. These non-cornified cells are doubtless more vulnerable to chemical

injury. At the same time inflammatory cells are called forth, probably a response to a leukotactic factor in the putative 'toxin'. These migrate through the epidermis, penetrate the ducts in quantity and eventually degenerate, co-mingling with the amorphous material to form dense impactions. After about 4 days of occlusion, the duct is completely obstructed corresponding to the time when anhidrosis reaches a peak in everyone. Then thermal stress will lead to extravasation of sweat through the damaged acrosyringium creating the clinical picture of miliaria rubra. The process of cell renewal leads eventually to sloughing of the impaction and restoration of sweating.

We would point out that this account differs considerably from past notions, not only in respect to the anatomical obstruction, but also with regards to a sub-clinical periductal inflammation prior to the onset of frank miliaria rubra. The escape of sweat into the tissue aggravates and perpetuates the pre-existing inflammation. The inflammatory change, theretofore unperceived, no doubt contributes to the weakening of the ductal cells increasing the likelihood of rupture when sweat distends the lumen. It should also be said that the term 'rupture' is not to be taken literally. A physical hole or gap cannot be demonstrated as after the blow-out of a tyre. What actually happens is a seeping of sweat through the damaged epithelial lining. We are currently trying to isolate the hypothetical bacterial 'toxin' from micrococci.

DISCUSSION

Miliaria rubra occurs spontaneously in certain persons when an intense heat load causes prolonged and profuse sweating. We think our observations provide an explanation for the origin of this common type of heat rash. Our hypothesis brings together diverse experimental observations, accounts for the obstruction to sweat delivery and establishes bacteria as aetiological. We shall summarize herewith the sequence of events in the development of miliaria rubra and our interpretation of these happenings.

Sweating hydrates the horny layer; this leads to a great increase in the population of resident aerobic bacteria. The microflora expands from a normal density of a few hundred organisms per cm^2 to hundreds of thousands and millions per cm^2 . This increase is an inevitable occurrence when the skin is covered with an impermeable dressing such as plastic film. Similar changes can occur during very hot weather under sweat-soaked clothing which then becomes a semi-occlusive dressing. We have in fact induced an expansion of the microflora and sparse miliarial lesions in a few active young males who wore their 'T' shirts continuously for 5 days during a summer hot spell. Substantial increases in the microflora of the glabrous skin can be brought about without complete occlusion. Duncan, McBride & Knox (1969) experimentally exposed volunteers to controlled environmental conditions for 60 h and noted an increase in the bacterial density by several orders of magnitude when temperature and humidity were high (90°F. , 95% R.H.). For instance, on the back the counts increased from tens or hundreds per cm^2 to thousands or tens of thousands. Similar changes occurred on other body areas as well. Multiple layers of even a loose material such as cotton gauze insulate the surface sufficiently to result in high local humidities thereby stimulating bacterial growth. Merely covering the skin under a plastic cup also can produce high bacterial densities, though not consistently. An impermeable film for 24 h produces only a temporary anhidrosis, which fades in an hour or so when the horny layer dries out. Even though the density of aerobes increases by orders of magnitude after 24 h of occlusion and does not increase thereafter it is only after 3 and more certainly after 4 days of continuous occlusion that an enduring anhidrosis is induced. It is this stable, long lasting anhidrosis which sets the stage for the development of miliaria. A durable sweat suppression was virtually undetectable after 1 day of occlusion and occurred in less than 50% after two. To achieve nearly complete anhidrosis in everyone required about 4 days. Thus, it is only after peak densities have been attained for several days that the phenomena of anhidrosis and miliaria can be regularly

induced. Diphtheroids and coagulase negative cocci dominate the normal aerobic bacterial microflora. Occlusion leads to an early and sharp increase of the cocci and it has been demonstrated by Marples & Kligman (1965) that diphtheroids lag behind in this expansion.

A number of findings pinpoint cocci as the *agents provocateurs*. Diphtheroids were recovered from only half the subjects. Thus, diphtheroids are not obligatory. One would have to create a pure diphtheroid microflora to ascertain whether these organisms can also induce anhidrosis. This holds for Gram negatives as well. On the other hand, yeasts can be perfunctorily excluded. Pure growths were sometimes encountered and yet the sites showed no signs of sweat suppression.

The definitive experiment for establishing a causative role for bacteria is to show that anhidrosis will not develop when the organisms are prevented from multiplying. Pretreatment with antibacterial substances is the appropriate device for accomplishing this. Even though fresh applications were made daily for 4 days, keeping the bacterial flora in check was more difficult than we had anticipated. With certain antibiotics, erythromycin and chloramphenicol for instance, resistant organisms emerged which spoiled the plan. Previous work by Marples & Kligman (1971) demonstrated that some antibiotics, penicillin for instance, are effective for only a short time because the native microflora rapidly acquires resistance. We suppose this is why Stillman *et al.* (1971) were led away from a bacterial hypothesis. Like ourselves, they were only partially successful in preventing miliaria with neomycin and chloramphenicol.

On the other hand, certain chemical bacteriostats, notably hexachlorophene and Mg-pyrithione, were highly effective in preventing the expansion of the microflora. With these the count was even less than on normal skin. Treated sites showed no sweat suppression whatever. The case for a bacterial aetiology, and especially for the specific role of cocci, was further strengthened by plotting the density of cocci against the degree of sweat suppression. Varying densities were achieved in two ways, by partially effective antibiotics and by coverings that hydrated the horny layer to different degrees. The regression line of density on hypohidrosis was strictly linear; the greater the number of bacteria the greater the sweat suppression.

A finding that was initially disconcerting was the lack of anhidrosis under water filled cups. We fully expected a great growth of organisms in this 'broth', supplied by skin nutrients. As it turned out, the result was entirely consistent with the bacterial hypothesis for the counts were very low, both on the surface and in the water itself.

Following the duration of anhidrosis after 4 days of occlusion proved to be highly informative. Griffin *et al.* (1967), Sulzberger *et al.* (1967) and Sulzberger & Griffin (1969) had found that it took 2-3 weeks for sweating to be fully restored after occlusion. We fully confirmed their findings. Some inhibition of sweating was still evident at 3 weeks. It cannot escape attention that the turnover time of the entire epidermis is about 4 weeks and that of the stratum corneum about half this amount. This suggests that the obstruction must be somewhere within the epidermis itself and is relieved by the process of cell renewal.

Histological studies provided the final evidence for integrating these findings into a unified concept. Experimenters have been consistently frustrated in identifying an obstruction in the early stage of anhidrosis. It will be noticed that our observations made no mention of the much vaunted 'horny plugs'. In reviewing the photos published by various authors, we deem the 'plugs' to be mainly normal structures. The terminal spirals of the duct wind through a 'beaker' of horny material; the latter is a substantial, impressive structure which is likely to be regarded as pathological unless one has examined many normal skin specimens.

After severe injury, the horny cells constituting the beaker may become parakeratotic; nucleated horny cells reflect the repair process and indicate the severity of the original injury, sometimes mentioned by other workers. Parakeratotic plugs surely do offer an obstruction to the flow of sweat,

probably explaining the anhidrosis in chronic dermatoses and after intense acute injury, viz., freezing, ultra-violet radiation, etc. In their recent investigation, Sulzberger & Harris (1972) could find no keratotic plugs nor any other kind of a block. Curiously we have had no difficulty in visualizing the formation of an anatomical obstruction. We regularly found an agglomerate of PAS-positive material and degenerating leukocytes which completely blocked the ducts. With repeated thermal stresses, the inflammatory reaction extended further downwards. Thereafter, as reparative processes came into play, the occluding mass moved upwards, finally occupying the coils of the ducts within the horny layer. These observations adequately explain the sequence which was sometimes observed, namely miliaria rubra, then profunda and finally crystallina.

The progressive sequence from rubra, to profunda and to crystallina is in fact characteristic of the heat rash which occurs spontaneously after repeated bouts of profuse sweating. By contrast, when anhidrosis is produced by protein precipitants, such as formalin or trichloroacetic acid, the obstruction is located in the pore (high level blockade) and heat rash then takes the form of miliaria crystallina as the first and characteristic expression. Deeper obstructions can be created by more severe insults in which case the first expression will be miliaria rubra.

If the events leading to miliaria rubra are as we have represented them, we can think of no satisfactory treatment for miliaria rubra except to avoid sweating. Lyons & Hunt (1955) and Lyons *et al.* (1962) claimed that topical antibiotics benefited miliaria rubra but it is hard to understand how this could occur since weeks are required to remove the obstructions. Perhaps the authors prevented the formation of new lesions. On the other hand pretreatment with antibacterial substances that will keep the microflora from expanding in hot, humid environments will almost certainly provide effective prophylaxis.

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REFERENCES

- ACTON, H.W. (1926) Prickly heat. *Indian Medical Gazette*, **61**, 321.
- BROWN, J.H. & BRENN, L. (1931) A method for the differential staining of Gram-pos. and Gram-neg. bacteria in tissue sections. *Bulletin of the Johns Hopkins' Hospital*, **48**, 69.
- CORMIA, F.E. & KUYKENDALL, V. (1954) Studies on sweat retention in various dermatoses. *Archives of Dermatology*, **69**, 543.
- DOBSON, R.L., LOBITZ, W.C. & HANOVER, N.H. (1957) Some histochemical observations on the human eccrine sweat glands: II. The pathogenesis of miliaria. *Archives of Dermatology*, **75**, 653.
- DUNCAN, W.C., MCBRIDE, M.E. & KNOX, J.M. (1969) Bacterial flora. The role of environmental factors. *Journal of Investigative Dermatology*, **52**, 479.
- GORDON, B.I. & MAIBACH, H.I. (1969) Adhesive tape anhidrosis. *Archives of Dermatology*, **100**, 429.
- GRIFFIN, T.B., MAIBACH, H.I. & SULZBERGER, M.B. (1967) Miliaria and anhidrosis. II. The relationship between miliaria and anhidrosis in man. *Journal of Investigative Dermatology*, **49**, 379.
- HAMBRICK, G.W. & BLANK, H. (1956) The microanatomy of miliaria crystallina. *Journal of Investigative Dermatology*, **26**, 327.
- HENNING, D.R., GRIFFIN, T.B. & MAIBACH, H.I. (1972) Studies on changes in skin surface bacteria in induced miliaria and associated hypohidrosis. *Acta dermato-venerologica*, **52**, 371.
- LYONS, R.E. & HUNT, J.A. (1955) The treatment of miliaria rubra with neomycin lotion. *Journal of Investigative Dermatology*, **24**, 557.
- LYONS, R.E., LEVINE, R. & AULD, D. (1962) Miliaria rubra, a manifestation of staphylococcal disease. *Archives of Dermatology*, **86**, 282.
- MARPLES, R.R. & KLIGMAN, A.M. (1965) The effect of hydration on the bacterial flora of the skin. In: *Skin Bacteria and their Role in Infection* (Ed. by H.I. Maibach and G. Hildick-Smith), p. 33. McGraw-Hill, New York.

- MARPLES, R.R. & KLIGMAN, A.M. (1969) Growth of bacteria under adhesive tapes. *Archives of Dermatology*, **99**, 107.
- MARPLES, R.R. & KLIGMAN, A.M. (1971) Ecological effects of oral antibiotics on the microflora of human skin. *Archives of Dermatology*, **103**, 148.
- NOBLE, W.C. (1975) Skin as a microhabitat. *Post Graduate Medical Journal*, **51**, 151.
- O'BRIEN, J.P. (1947) A study of miliaria rubra, tropical anhidrosis and anidrotic asthenia. *British Journal of Dermatology*, **59**, 125.
- O'BRIEN, J.P. (1950) The aetiology of poral closure. I-IV. *Journal of Investigative Dermatology*, **15**, 95.
- ORENTEICH, N., BERGER, R.A. & AUERBACH, R. (1966) Anhidrotic effects of adhesive tapes and occlusive film. *Archives of Dermatology*, **94**, 709.
- PAPA, C.M. & KLIGMAN, A.M. (1966) Mechanisms of eccrine anhidrosis. I. High level blockage. *Journal of Investigative Dermatology*, **47**, 1.
- POLLITZER, S. (1893) Prickly heat—lichen tropicus, miliaria papulosa, miliaria rubra, etc. *Journal of Cutaneous and Genito-Urinary Diseases*, **11**, 50.
- RANDALL, W.C. & PEISS, C.N. (1957) The relationship between skin hydration and the suppression of sweating. *Journal of Investigative Dermatology*, **28**, 435.
- ROBINSON, A.R. (1884) Miliaria and sudamina. *Journal of Cutaneous and Venereal Diseases*, **2**, 362.
- SARKANY, I. & GAYLARDE, P. (1968) A method for demonstrating of sweat gland activity. *British Journal of Dermatology*, **80**, 601.
- SARKANY, I., SHUSTER, S. & STAMMERS, M.C. (1965) Occlusion of the sweat pore by hydration. *British Journal of Dermatology*, **77**, 101.
- SHELLEY, W.B. & HORVATH, P.N. (1950a) Experimental miliaria in man. II. Production of sweat retention anhidrosis and miliaria crystallina by various kinds of injury. *Journal of Investigative Dermatology*, **14**, 9.
- SHELLEY, W.B. & HORVATH, P.N. (1950b) Experimental miliaria in man. III. Production of miliaria rubra (prickly heat). *Journal of Investigative Dermatology*, **14**, 193.
- SINGH, G. (1973) Role of bacteria in anhidrosis. *Dermatologica*, **146**, 256.
- STILLMAN, M.A., HINDSON, T.C. & MAIBACH, H.I. (1971) The effect of pretreatment of skin on artificially induced miliaria rubra and hypohidrosis. *British Journal of Dermatology*, **84**, 110.
- SULZBERGER, M.B. & EMIK, L.O. (1946) Studies on prickly heat: I. Clinical and statistical findings. *Journal of Investigative Dermatology*, **7**, 53.
- SULZBERGER, M.B. & GRIFFIN, T.B. (1969) Induced miliaria, post miliarial hypohidrosis and some potential sequelae. *Archives of Dermatology*, **99**, 145.
- SULZBERGER, M.B., GRIFFIN, T.B. & WILEY, H.S. (1967) Miliaria and anhidrosis. I. Experimental production in volunteers. *Dermatologica*, **135**, 414.
- SULZBERGER, M.B. & HARRIS, D.R. (1972) Miliaria and anhidrosis. III. Multiple small patches and the effects of different periods of occlusion. *Archives of Dermatology*, **105**, 845.
- SULZBERGER, M.B. & ZIMMERMAN, H.M. (1946) Studies on prickly heat. II. Experimental and histological findings. *Journal of Investigative Dermatology*, **7**, 61.
- TIMMERMAN, J.C., LONG, J.P. & PITTINGER, C.B. (1959) Neuromuscular blocking properties of various antibiotic agents. *Toxicology and Applied Pharmacology*, **1**, 299.
- UNNA, P.G. (1896) *The Histopathology of Diseases of the Skin*, p. 908. William F. Clay, Edinburgh.
- WILLIAMSON, P. & KLIGMAN, A.M. (1965) A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology*, **45**, 498.
- WILLIS, I. (1973) The effects of prolonged water exposure on human skin. *Journal of Investigative Dermatology*, **60**, 166.

Epidermal differentiation in normal and growth-retarded infants: studies in two animal models and in human babies

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SUMMARY

The influence of prenatal growth retardation on epidermal growth and keratinization was studied in small-for-dates human babies, runt piglets and in rat fetuses subject to maternal protein deprivation. In the human babies and rat fetuses growth retardation was associated with reduced epidermal growth but normal patterns of differentiation were present. Thus, epidermal thickness was less and keratinizing zones narrower than seen in normal weight individuals. In the runt (small-for-dates) piglet, epidermal development differed from that seen in normal weight piglets of the same gestational age and from that seen in the human and rat. The epidermis was thicker with a negligible stratum granulosum and with the stratum corneum containing nucleated cells, a condition resembling 'parakeratosis'. This pattern was less often seen in normal weight piglets.

Differences between the skins of the three species studied at a perinatal stage may be related to clear dissimilarities which exist in the tissues later. Parakeratosis as a transitory phase in the development of pig skin is probably related to a higher rate of epidermal keratinization in this species than occurs in either human babies or rat fetuses.

In the mammalian epidermis, mitotic activity and keratinocyte differentiation are determined by a complex interaction between environmental factors and intrinsic control mechanisms, notably chalone and hormones (Bullough & Laurence, 1960, 1964; Bullough, 1965; Marks, 1972). Less information is available concerning epidermal development prenatally and its regulation. Presumably, once epidermal cells have attained a certain critical level of differentiation from the embryonic ectoderm, they will be subject to those control mechanisms present in the post-natal state. However, in the fetus the control of environmental factors is largely a function of the mother and is thus dependent upon her age, genetic status and general state of health. Histochemical studies indicate that developmental changes in fetal skin are related to constitutional changes in amniotic fluid, particularly

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its glycogen content (Hoyes, 1968; Moynahan, Sethi & Brookes, 1972). From this it follows that a condition of maternal ill-health as in malnutrition, resulting in pathological alterations in the amniotic fluid and anomalous fetal development, would be expected to impair epidermal development also. Experimentally, where maternal ill-health was induced by Cocksackievirus B3 infection, fetal growth was retarded and differentiation in the epidermis delayed (Lansdown, 1975). In these fetuses, epidermal development was consistent with body weight and not gestational age.

The present study further examines the relationship between fetal growth impairment and retardation and epidermal differentiation using two animal models other than Cocksackievirus infection in mice. Epidermal growth and keratinization seen in these studies was compared to that seen in human babies of normal and sub-normal weight who died perinatally at Northwick Park or Mayday (Croydon) Hospitals.

Animal models

(a) *Dietary protein deprivation in pregnant rats.* Pregnant Sprague-Dawley rats weighing approximately 250 g were fed a standard rodent diet containing 5% w/w protein (casein) from day 1 of pregnancy. Control animals were given a Spillers small rodent diet (No. 1) containing approximately 21% total protein. Four animals were used in each group and in each case were killed on the 22nd day of pregnancy with excess Nembutal anaesthetic. Representative samples of back skin were taken from the interscapular region of 40 fetuses. Skin pieces 20 mm² were fixed in 10% neutral phosphate buffered formalin or in 70% alcohol for histological examination. Sections 5–7 µm thick were routinely stained by haematoxylin and eosin, periodic acid Schiff (PAS) technique for glycogen, Baker's (1944) method for phospholipid, and by the congo red/thioflavine T method for epidermal keratin (Jarrett, Bligh & Hardy, 1956). Keratohyalin granules in the stratum granulosum were demonstrated by titan yellow fluorescence.

(b) *Spontaneous intrauterine growth retardation in pigs* (Widdowson, 1971). In pigs there is frequently a reduction in fetal body weight from the ovarian end towards the centre of the uterus, the smaller piglets being known as 'runts'. They may weigh 50% or less than normal piglets at birth and are developmentally retarded (Cooper, 1973). Eight time-mated sows were anaesthetized at 100 days after mating and the young delivered by Caesarian section. Each was weighed and 40 mm² samples of interscapular back skin prepared for histology as described above for the rat fetus skin.

Human baby skin

Samples of skin were obtained from the interscapular region of fifteen babies that died perinatally or within the first 5 days of life. The skins were fixed in 10% neutral formalin or in 70% alcohol and histological sections stained as above for the animal tissues.

RESULTS

Epidermal development in fetal skin from rats fed normal or low protein diet during pregnancy

Rat fetuses from the low dietary protein group were significantly smaller than those from the control group ($P < 0.001$). The average body weight for the growth-retarded fetuses was 3.90 g compared to 5.27 g for normal 22-day individuals.

Interscapular back skin in normal rat fetuses consisted of a clearly distinguishable stratum germinativum (basal layer) composed of low columnar cells with strongly basophilic nuclei; a stratum spinosum consisting of 3–5 layers of irregularly shaped cells showing a tendency to be flattened in a plane

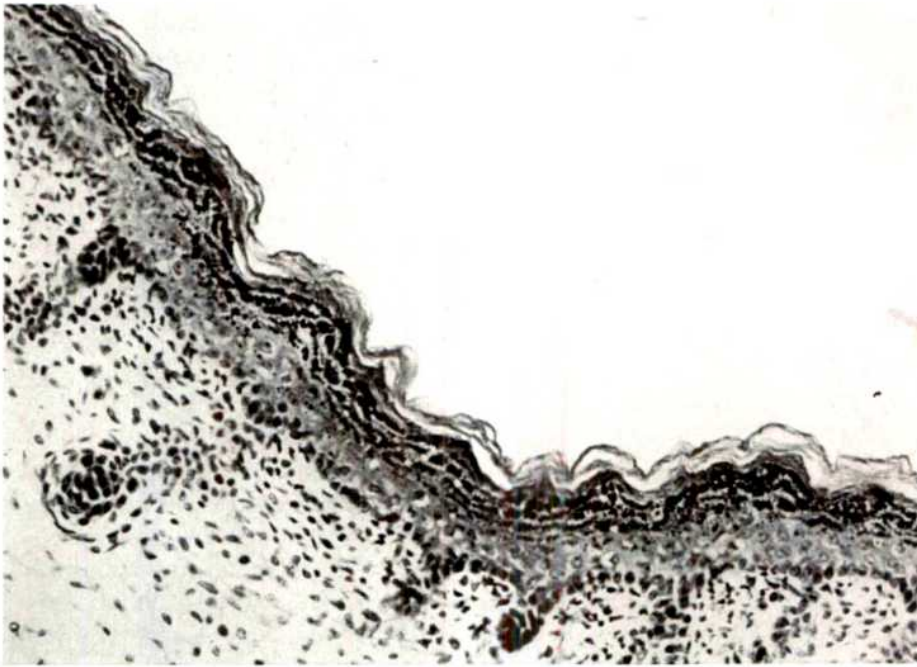


FIGURE 1. Interscapular back skin from a normal rat fetus of 22-days gestation showing epidermal keratinizing zones (H & E, $\times 16$).



FIGURE 2. Interscapular back skin from a growth-retarded rat fetus of 22-days gestation from a mother fed low dietary protein. Note reduced thickness of epidermal cell layers (H & E, $\times 16$).

parallel with the skin surface; a multilaminar stratum granulosum and a compact, homogeneously staining stratum corneum (Fig. 1). Keratohyalin granules in the stratum granulosum varied in size with the larger granules more numerous in those cells immediately below the stratum corneum. This latter layer fluoresced red with congo red stain, and keratohyalin yellow with titan yellow staining. Phospholipid deposition was seen in the keratohyalin granules and in hair root and sheath cells but rarely in the stratum corneum.

Fetal growth retardation in rats through maternal protein deprivation was associated with reduced epidermal thickness and a less clear demarcation between the stratum germinativum and stratum spinosum (Fig. 2). Keratohyalin granules were less numerous but up to 4 layers were present in some fetuses. Cells in the stratum spinosum and granulosum were less elongated than normal but in other respects did not differ morphologically from normal. Phospholipid distribution and keratinization were similar but less developed than in normal-weight fetuses.

Epidermal development in normal and runt (small-for-dates) piglets

At approximately 100 days gestation, fetal pig weights ranged from 250 to 750 g. For comparative purposes, they were grouped according to body weight: (A) Less than 400 g; (B) 401–600 g; (C) greater than 600 g. In fetuses of groups B and C, the epidermis exhibited a compact stratum corneum, a stratum spinosum of 3–5 layers, and a single layer of stratum germinativum composed of low columnar cells (Fig. 3). In general, the stratum corneum was narrower than the Malpighian layer and was separated from it by a narrow band of keratohyalin granules showing a mild titan yellow fluorescence. There was no distinct stratum granulosum as seen in fetal rats. On occasions, nuclear debris was present in the stratum corneum in the form of a parakeratosis. Phospholipid was identified as an

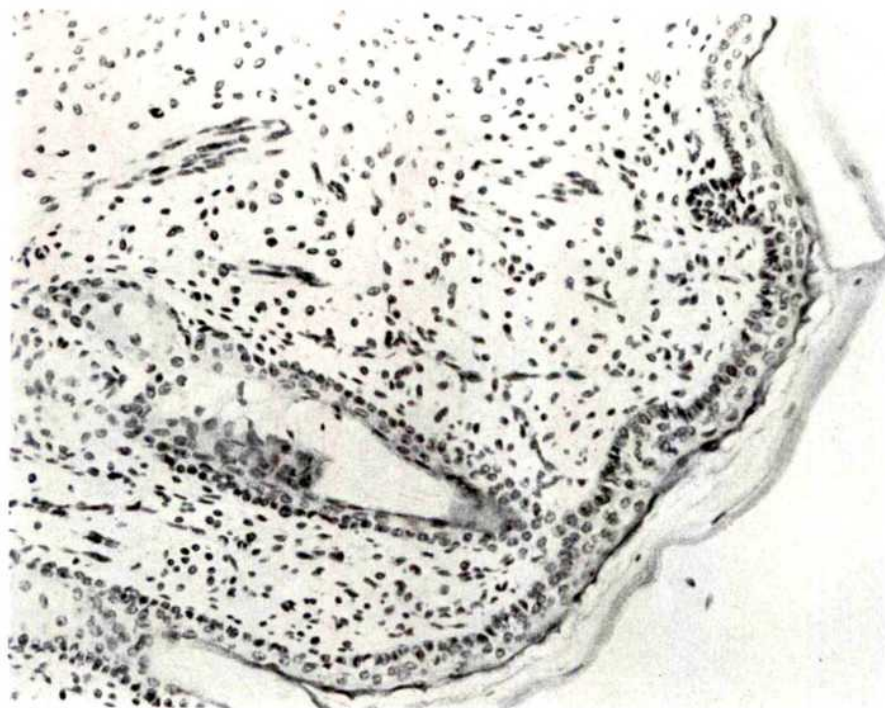


FIGURE 3. Epidermal differentiation in a normal weight piglet at 100-days gestation (H & E, $\times 16$).

outer band in the stratum corneum and in the hair root and sheath cells as well as in keratohyalin granules. Keratin in the stratum corneum routinely fluoresced with a dull blue-grey colour and not the bright red colour as seen in adult tissues.

Low fetal weight in runt piglets was characterized by a thick stratum corneum of loosely packed cells many of which contained nuclear debris (Fig. 4). The stratum corneum was at least as thick as the Malpighian layer consisting of a basal layer and 3-5 layers of stratum spinosum cells, and was separated from it by an indistinct band of keratohyalin granules as described above. Phospholipid distribution and keratin fluorescence in runt piglets resembled that seen in group B and C piglets.

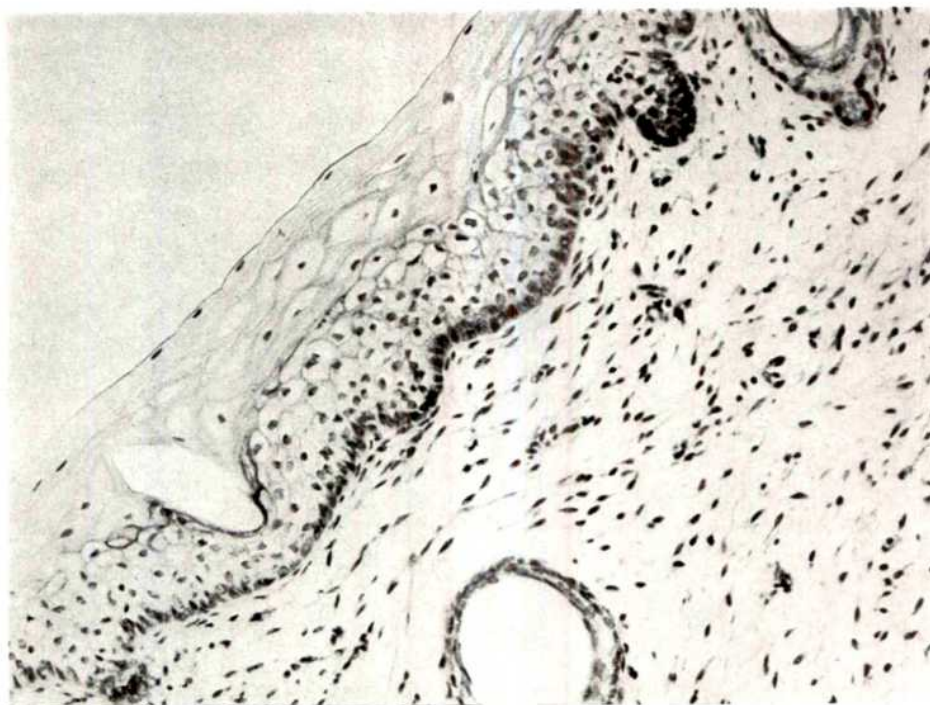


FIGURE 4. Epidermal differentiation in a small-for-dates pig fetus of 100-days gestation showing nucleated stratum corneum and minimal keratohyalin granulation (H & E, $\times 16$).

Epidermal development in human babies

Of the 15 babies from whom skin samples were available for histological examination, three weighed more than 3000 g at autopsy and the others were of less than 2000 g. Four were stillborn, five died as a consequence of extreme prematurity and failure to thrive. In one child, death was associated with congenital deformities of spina bifida and exencephaly (birthweight 3050 g). One baby (3570 g) died as a result of pulmonary congestion resulting from inhalation of gastric contents. For the other babies, the cause of death was not available. Available information on all babies examined did not show metabolic defects which might affect their skin development.

In the babies examined, difficulty was experienced in obtaining good representative material for histology. Several of the infants dying shortly before birth exhibited a marked deterioration of the epidermis with advanced desquamation of the stratum corneum and autolytic changes in the Malpighian layer. However, available material indicated that the epidermis in the normal weight human

baby within 48 h of birth is a four-layered structure composed of a stratum corneum, stratum granulosum, stratum spinosum and stratum germinativum. In each case, the keratin fluoresced red with congo red dye but did not contain demonstrable phospholipid which was only present in keratohyalin granules. The stratum spinosum comprised 3–5 layers of irregularly shaped cells with a rich content of glycogen. The stratum germinativum was not clearly distinguished from the stratum spinosum and cells were identified more by their location than by their morphological criteria.

Growth retardation in human babies was consistent with delayed or impaired differentiation in the epidermis. For example, in two babies weighing less than 1000 g at birth, the epidermis was exceptionally thin with a very narrow keratin layer and a discontinuous stratum granulosum. Nuclear debris was occasionally present in the keratinized tissue. Glycogen was not plentiful in epidermal cells. The Malpighian layer varied greatly in thickness from 1–2 cell layers in very small babies to 3–4 layers in larger individuals (Fig. 5). Congo red fluorescence in the keratin varied from a uniform red colouration to a dull blue-grey in outer keratinized cells. Phospholipid deposition was similar to that identified in larger babies but the intensity of the staining reaction was less.

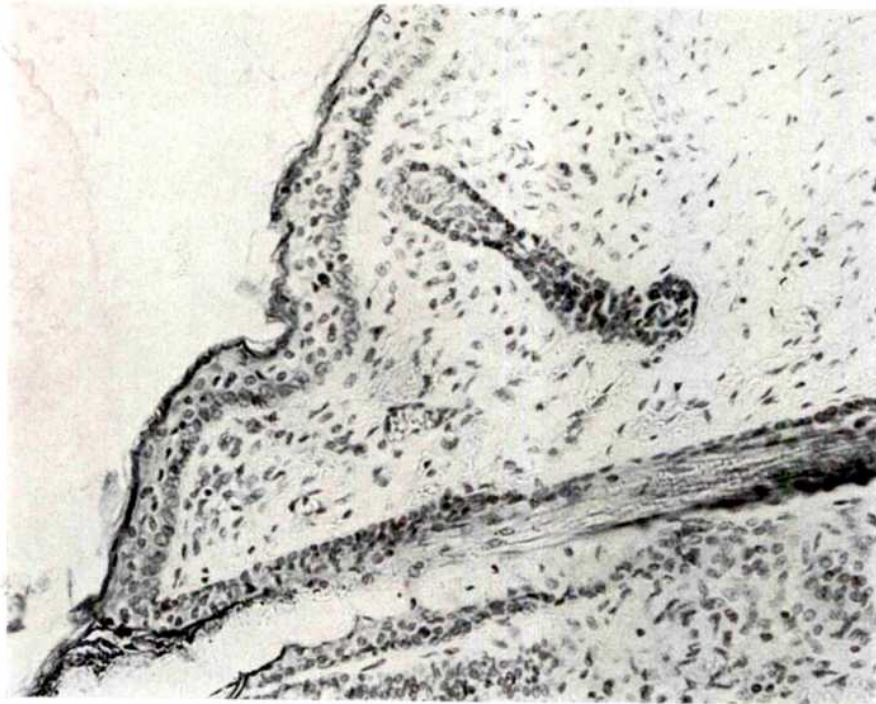


FIGURE 5. Interscapular back skin from a small-for-dates human infant dying within 4 h of birth (birthweight 1050 g). Note minimal stratum corneum (H & E, $\times 16$).

DISCUSSION

In fetal mammals, the skin is derived from the embryonic ectoderm which gives rise to the periderm and cell layers present in adult skin (Breathnach & Robbins, 1969; Hanson, 1947). In a particular species, the rate and pattern of differentiation in fetal skin is related to the region of the body, sex, the duration of the gestational period and the birthweight. Clinical studies in human babies have

shown a clear correlation between skinfold thickness and birth weight but not fetal maturity (Farr, 1966; Gampel, 1965; Hutchinson-Smith, 1973).

Two main features emerge from the present comparative study of the epidermis in normal and growth-retarded offspring. Firstly, in normal weight individuals in the three species examined, considerable differences exist in the keratinizing zones of the epidermis. Secondly, prenatal growth retardation in human babies and in rat fetuses, and possibly in piglets also, is associated with a normal but delayed differentiation pattern. Thus all the main layers of the epidermis are present but the differentiation is less advanced than would be expected for the gestational age. The histological profiles are probably similar to those seen at earlier stages in normal intrauterine growth.

The variations between the species in patterns of keratinization perinatally may reflect differences in epidermal morphology which are evident later in life. Human back skin in adulthood is of smooth texture and is sparsely covered with hair, pig skin is thickened and rough in appearance with a coarse hair cover, whereas in rats the integument is densely haired and the epidermis correspondingly thin with minimal keratinization (Montagna, 1972). The nature of the keratinization process and the role of keratohyalin granules has been the object of many light and electron microscopic studies. It is now clear that keratohyalin is a sulphur-rich amorphous precursor of the content of keratinized cells (Wier, Fukuyama & Epstein, 1971; Ugel, 1971; Lavker & Matoltsy, 1970), and not a by-product of the keratinization process (Matoltsy & Matoltsy, 1970). Several layers of keratohyalin granules in fetal rat skin may thus indicate a prolonged keratinization process compared to that seen in the human baby or piglet skin, where keratohyalin granules are fewer and at the most arranged in a single narrow band. The congo red fluorescence in the stratum corneum in human baby and fetal rat skin resembling that seen in adults suggests that in these species the keratin formed perinatally is of a 'complete' form. In contrast, in piglet skins where the keratin does not fluoresce as in the adult and where keratohyalin granules are not plentiful, it would seem that the keratin formed is rapid and 'incomplete'. Epidermal cells thus pass from the stratum spinosum stage into the keratinized state with an abbreviated keratohyalin-containing stage. The condition resembles the parakeratosis as occurs in the rodent tail and in mouse skin following the application of mild irritants (Lansdown & Grasso, 1972; Lansdown, 1973). Further, since in adulthood pig skin exhibits a well defined stratum granulosum and a thick stratum corneum, it seems that this parakeratotic phase is a transitory stage in normal development. The full significance of the parakeratosis in normal development is not clear but it is no doubt related to the attainment of the characteristically thick skin of this species.

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REFERENCES

- BAKER, J.R. (1944) The structure and chemical composition of the Golgi element. *Quarterly Journal of Microscopical Science*, **87**, 441.
- BREATHNACH, A.S. & ROBBINS, J. (1969) Ultrastructural features of epidermis of a 14 mm (6 week) human embryo. *British Journal of Dermatology*, **81**, 504.
- BULLOUGH, W.S. (1965) Mitotic and functional homeostasis. *Cancer Research*, **25**, 1683.
- BULLOUGH, W.S. & LAURENCE, E.B. (1960) The control of mitotic activity in the mouse skin. *Experimental Cell Research*, **21**, 394.

- BULLOUGH, W.S. & LAURENCE, E.B. (1964) Mitotic control by internal secretions: the role of the chalone: adrenalin complex. *Experimental Cell Research*, **33**, 176.
- COOPER, J.E. (1973) The use of the pig as an animal model to study problems associated with low birthweight. *Laboratory Animals*, **9**, 329.
- FARR, V. (1966) Skinfold thickness as an indication of maturity in the newborn. *Archives of Diseases in Childhood*, **41**, 301.
- GAMPEL, B. (1965) The relation of skinfold thickness in the neonate to sex, length of gestation, size at birth and maternal skinfold. *Human Biology*, **37**, 29.
- HANSON, J. (1947) The histogenesis of the epidermis in the rat and mouse. *Journal of Anatomy*, **81**, 174.
- HOYES, A.D. (1968) Electron microscopy of the surface layer (periderm) of human foetal skin. *Journal of Anatomy*, **103**, 321.
- HUTCHINSON-SMITH, B. (1973) Skinfold thickness in infancy in relation to birth weight. *Developmental Medicine and Child Neurology*, **15**, 628.
- JARRETT, A., BLIGH, A. & HARDY, J. (1956) Fluorescent microscopy of the human skin. *British Journal of Dermatology*, **71**, 277.
- LANSDOWN, A.B.G. (1973) Production of epidermal damage in mammalian skins by simple aluminium compounds. *British Journal of Dermatology*, **89**, 67.
- LANSDOWN, A.B.G. (1975) Histological observations on epidermal development in foetal mice subject to intrauterine growth retardation due to maternal infection with Coxsackievirus B3. *Biology of the Neonate*, **27**, 368.
- LANSDOWN, A.B.G. & GRASSO, P. (1972) Physico-chemical factors influencing epidermal damage by surface active agents. *British Journal of Dermatology*, **86**, 36.
- LAVKER, R.M. & MATOLTSY, A.G. (1970) Formation of horny cells. The fate of cell organelles and differentiation products in ruminal epithelium. *Journal of Cell Biology*, **44**, 501.
- MARKS, R. (1972) The role of chalones in epidermal homeostasis. *British Journal of Dermatology*, **86**, 543.
- MATOLTSY, A.G. & MATOLTSY, G. (1970) The chemical nature of keratohyalin granules of the epidermis. *Journal of Cell Biology*, **47**, 593.
- MONTAGNA, W. (1972) The skin of non-human primates. *American Zoologist*, **12**, 109.
- MOYNAHAN, E.J., SETHI, N.C. & BROOKES, M. (1972) Histochemical observations on developing rat skin. *Journal of Anatomy*, **131**, 427.
- UGEL, A.R. (1971) Studies on isolated aggregating oligoriboproteins of the epidermis with histochemical and morphological characteristics. *Journal of Cell Biology*, **49**, 405.
- WIDDOWSON, E.M. (1971) Intrauterine growth retardation in the pig, I. Organ size and cellular development at birth and after growing to maturity. *Biology of the Neonate*, **19**, 329.
- WIER, K.A., FUKUYAMA, K. & EPSTEIN, W.L. (1971) Nuclear changes during keratinization of the normal human epidermis. *Journal of Ultrastructure Research*, **37**, 138.

Physical and chemical properties of skin in habitually trained and sedentary men

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SUMMARY

A number of physical and chemical properties of skin were examined in a study of 29 habitually trained and 29 sedentary men. Compared to the control group, the trained subjects had significantly higher values in the weights of skin samples of equal surface areas as well as the contents of hydroxyproline and nitrogen per skin surface area. When measured by a diaphragm method *in vivo*, the 'elastic stiffness' (uncorrected for thickness) and 'elastic efficiency' (the recovery of the deformation energy) of skin were significantly higher in the trained men compared to those in the untrained men. The results suggest that skin reflects the adaptation to habitual endurance training by increasing its mass and strengthening its structure. The study did not, however, reveal any differences between physically active and sedentary men in changes due to biological ageing.

Previous animal experiments have shown that physical training has a significant influence on the connective tissues of the locomotor system (Booth & Gould, 1975). Only scanty scientific data are, however, available on the effects of training on the connective tissue of skin. Heikkinen & Vuori (1972) observed a slight retardation in the metabolism of collagen in the skin of aged inactive mice. Simultaneously, they found increased concentrations of hexosamines and nitrogen in the skin of physically active mice. According to the recent results of Kiiskinen & Heikkinen (1976) physical training also increases the concentration of skin hydroxyproline, hexosamines and nitrogen in growing mice. As far as we know, no reports have been published on the effects of 'lifelong' endurance training on the properties of human skin.

On the other hand, changes in skin with ageing are well known, e.g. the concentration of collagen increases, whereas the collagen biosynthesis, the acid-soluble collagen and the concentration of ground substance glycosaminoglycans decrease with advancing age (Bakerman, 1962; Heikkinen, 1968; Lindner, 1972; Uitto, 1971). The mechanical stiffness of skin becomes more pronounced and the toe part of its stress-strain curve diminishes while its tensile strength increases (Viidik, 1973a, 1973b). *In vivo* experiments on human subjects have also shown a considerable loss of elasticity

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(Hollingsworth, Hashizume & Jablon, 1965) and increasing elastic modulus (Grahame, 1970) with ageing.

The purpose of the present investigation was to study some of the physical and chemical properties of skin in middle-aged and elderly endurance athletes in habitual training in comparison with those in sedentary men of similar ages.

MATERIAL AND METHODS

Subjects and experimental design

Two separate experiments were performed. Twenty-nine male endurance athletes, aged 32–69 years, volunteered for experiment I. The majority of them had been in regular training (running and/or cross-country skiing at an average of 50 km per week) throughout their adult life. The trained men were matched with 29 healthy untrained control subjects of similar ages (31–68 years). Twenty-one men from both the trained and sedentary group (aged 34–70 years and 33–69 years, respectively) participated in experiment II 1 year later. The latter experiment was performed as a part of a larger study, the other results of which have been published elsewhere (Suominen & Heikkinen, 1975; Suominen *et al.*, 1978). The reduction in subjects from experiment I to experiment II was partly due to the matching procedure, i.e. if one man from either group could not participate, his counterpart was also removed. Of the athletes who were removed before experiment II, four had stopped regular training and one left the neighbourhood. Of the sedentary subjects, one had died, one had coronary heart disease and one had started physical training. The physical characteristics of the subjects appear in Table 1.

TABLE 1. Physical characteristics of subjects. Number of pairs and mean $\pm$ s.d. are given

Variable	Experiment	Trained	Sedentary
Age (years)	I (29)	47.2 $\pm$ 10.1	47.1 $\pm$ 10.2
	II (21)	49.1 $\pm$ 10.2	48.7 $\pm$ 10.2
Height (cm)	I (29)	171.9 $\pm$ 6.5	173.6 $\pm$ 5.6
	II (21)	170.1 $\pm$ 6.9	171.8 $\pm$ 6.2
Weight (kg)	I (29)	68.0 $\pm$ 7.2*	74.6 $\pm$ 10.0
	II (21)	67.1 $\pm$ 6.8	72.3 $\pm$ 11.1

* Significantly different ($P < 0.01$) from the value of the sedentary men.

Skin biopsies (experiment I)

In each subject, two skin biopsies were obtained with a 4 mm punch from the medial part of the brachium under local anaesthesia. The biopsy specimens were immediately trimmed of subcutaneous tissue, weighed with an automatic analytical balance (Mettler H20T) and stored at -23°C for subsequent chemical analyses.

Chemical analyses

The first skin sample was dried to a constant weight at $+95^{\circ}\text{C}$ and hydrolyzed in 2 M HCl for 16 h at $+100^{\circ}\text{C}$. A part of the hydrolyzate was used for the determination of hexosamines (Gatt & Berman, 1966). The remainder was further hydrolyzed in 6 M HCl for 3 h at $+130^{\circ}\text{C}$. From the hydrolyzate, the concentration of hydroxyproline was assayed by the colour reaction of Stegeman as modified by

Woessner (1961) and that of nitrogen according to Minari & Zilversmit (1963) after combustion of the sample in 8 M H_2SO_4 .

The second skin sample was homogenized five times for 5 s in 1 ml of 0.5 M acetic acid by using a Potter-Elvehjem-type homogenizer kept in an ice bath. The homogenate was incubated in a metabolic shaker for 30 min at $+40^\circ\text{C}$ and centrifuged at 20,000 rev/min for 30 min at $+20^\circ\text{C}$. The extraction procedure was repeated twice (Heikkinen, 1968). Both the sediment and combined supernatants were lyophilized, hydrolyzed in 6 M HCl for 3 h at $+130^\circ\text{C}$ and analyzed for hydroxyproline and nitrogen as above. The percentage of supernatant hydroxyproline from the total (sediment + supernatant) hydroxyproline was taken as a measure of collagen soluble in 0.5 M acetic acid. Similarly, the nitrogen analyses were used to estimate total soluble protein.

The water content of skin was determined from the difference between the wet and dry weight of the first biopsy specimen.

Mechanical analyses (experiment II)

The mechanical properties of the skin of the ventral side of the upper third of the forearm were measured *in vivo* by a diaphragm method (Dick, 1951; Grahame, 1970) using an apparatus constructed in our laboratory. The structure and the functional principle of the apparatus are schematically presented in Fig. 1. The apparatus consisted of two piston pumps connected to a special 'suction cup', the inner

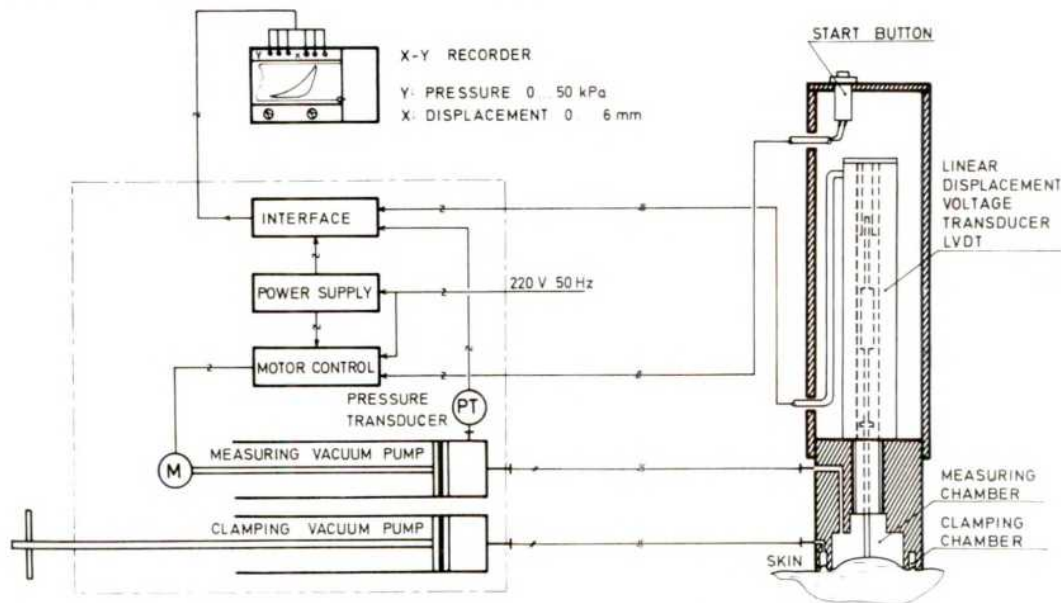


FIGURE 1. Schematic presentation of measuring apparatus of mechanical properties of skin.

diameter of which was 2 cm. A hand-operated piston pump was used to produce the vacuum needed for clamping the skin around the test area. After pushing the start button, a motor-driven vacuum pump produced the standard negative pressure applied in the stretching of the intact skin diaphragm. The measuring cycle (raising and lowering the pressure at a constant speed) lasted 31 s. An interface unit converted the voltages, which were proportional to the pressure and displacement, to appropriate units: pressure range 0–50 kPa or 5 kPa/cm and displacement range 0–6 mm or 0.5 mm/cm. An X-Y recorder (Minigor RE 501, X: 20 mV/cm, Y: 50 mV/cm) plotted the load-deformation curve

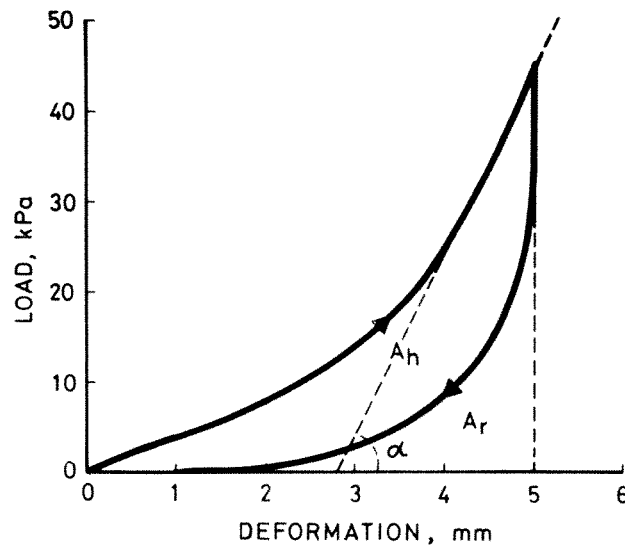


FIGURE 2. Typical load-deformation curve in measuring mechanical properties of skin. α = angle between linear region and deformation axis; A_r = area representing recovered deformation energy; A_h = area representing nonrecovered deformation energy (hysteresis loss).

(Fig. 2.). The following two parameters were calculated from the curve: (1) 'elastic stiffness' expressed as tangent α of the angle of the linear region of the rising curve and the X-axis (Viidik, 1973a) and (2) 'elastic efficiency' expressed as the percentage of the recovered energy (surface area A_r) from the total energy (surface area $A_r + A_h$) used for the deformation of the skin (see Fig. 2.). The surface areas were measured with an automatic optical planimeter constructed in the Department of Biology of Physical Activity, University of Jyväskylä. Including the results of some later experiments, the coefficient of variation for duplicate measurements made in the adjacent sites of the forearm was $\pm 4.7\%$ for 'elastic stiffness' and $\pm 5.7\%$ for 'elastic efficiency'.

Statistical methods

Standard procedures were used to calculate means and standard deviations (s.d.). The correlation coefficients (Pearson r) between chronological age and the skin variables were also determined. The statistical significance were calculated by Student's 't'-test (2P) for non-correlating means and for correlation coefficients.

RESULTS

Table 2 shows the results of the variables analysed from the skin biopsies in experiment I. Both the wet and dry weights of the skin samples of equal surface areas were significantly higher for physically active than sedentary men. Compared to the control group, the trained subjects also had significantly more hydroxyproline and nitrogen per surface area of the skin biopsy. No significant differences between the two groups, however, existed in the concentrations of these variables, i.e. when the results were expressed per wet or dry weight of the skin sample. Neither did the other chemical variables differ significantly between physically active and sedentary men.

The results of the mechanical analyses are shown in Table 3. Both the 'elastic stiffness' and 'elastic

TABLE 2. Comparison of different skin biopsy variables in habitually trained and sedentary men in experiment I. Mean $\pm$ s.d. is given (n=29 pairs, except 23 for solubility measurements)

Variable	Trained	Sedentary
Biopsy wet weight (mg)	6.20 $\pm$ 0.90*	5.57 $\pm$ 0.92
Biopsy dry weight (mg)	1.97 $\pm$ 0.34†	1.73 $\pm$ 0.26
Hydroxyproline (μ g/mm ²)	15.2 $\pm$ 2.9†	13.0 $\pm$ 2.6
(μ g/mg w.w.)	30.7 $\pm$ 3.5	29.4 $\pm$ 3.5
(μ g/mg d.w.)	96.8 $\pm$ 6.8	94.5 $\pm$ 8.4
Hexosamine (μ g/mm ²)	0.800 $\pm$ 0.118	0.741 $\pm$ 0.127
(μ g/mg w.w.)	1.65 $\pm$ 0.16	1.68 $\pm$ 0.20
(μ g/mg d.w.)	5.17 $\pm$ 0.65	5.40 $\pm$ 0.62
Nitrogen (μ g/mm ²)	24.1 $\pm$ 5.2†	20.7 $\pm$ 4.3
(μ g/mg w.w.)	48.8 $\pm$ 6.6	46.8 $\pm$ 6.6
(μ g/mg d.w.)	154 $\pm$ 16	150 $\pm$ 19
Hexosamine/hydroxyproline	0.0536 $\pm$ 0.0073	0.0577 $\pm$ 0.0087
Nitrogen/hydroxyproline	1.59 $\pm$ 0.16	1.59 $\pm$ 0.17
Soluble collagen (%)	6.43 $\pm$ 3.54	7.84 $\pm$ 3.89
Total soluble protein (%)	21.5 $\pm$ 6.0	22.9 $\pm$ 6.0
Water content (%)	68.3 $\pm$ 2.3	68.8 $\pm$ 2.5

*† Significantly different ($P < 0.05$, $P < 0.01$, respectively) from the value of the sedentary men.

TABLE 3. Comparison of mechanical properties of skin in habitually trained and sedentary men in experiment II. Mean $\pm$ s.d. is given (n=21 pairs)

Variable	Trained	Sedentary
Elastic stiffness (tan α) (kPa/mm)	21.5 $\pm$ 2.1*	19.4 $\pm$ 2.1
Elastic efficiency (%)	35.8 $\pm$ 3.9*	31.2 $\pm$ 4.6

* Significantly different ($P < 0.01$) from the value of the sedentary men.

efficiency' of skin were significantly higher in the trained men compared to those in the untrained men.

As judged from the correlation coefficients, no significant age-related changes in the skin variables occurred in the two groups of the study.

DISCUSSION

The results of the present study show that physical training affects the properties of human skin. Unlike the results of experiments in mice (Kiiskinen & Heikkinen, 1976) the athletes did not, however, demonstrate any higher concentrations of the different chemical variables in comparison with the sedentary men. It is possible that certain extraneous factors, e.g. subcutaneous fat, have influenced the concentration values in the above study. Yet the higher weights of skin samples as well as higher contents of hydroxyproline and nitrogen per skin surface area in the trained men indicate that a hypertrophy of skin occurs as a result of habitual endurance training. Hypertrophy-like changes and/or enhanced metabolism of various connective tissues especially in the locomotor system after

prolonged physical training have been shown earlier in animal studies (Booth & Gould, 1975). Although the corresponding empirical evidence in human subjects is scanty, one might assume that skin to a certain degree reflects the training-induced changes of the connective tissues which are directly loaded in physical work. On the other hand, it is obvious that many functions of skin itself are subjected to dramatic metabolic changes during exercise. It is possible that skin adapts to increased physiological stress by increasing its mass and strengthening its structure. The mechanisms of the observed skin hypertrophy, the role of hormonal factors, etc. could not, however, be clarified in the present study.

The typical age-related changes in the chemical properties of human skin (Bakerman, 1962; Clausen, 1962, 1963; Fleischmajer, Perlsh & Bashey, 1972; Sobel *et al.*, 1958) were not found in our subjects ranging in age from 31 to 69 years. Therefore, it is quite difficult to compare the 'biochemical ageing' of skin between physically active and sedentary men. The present results give no evidence of the possibility of counteracting the chemical ageing changes in skin by habitual training. Compared to the control group, the athletes had rather lower values, e.g. in the hexosamine/hydroxyproline ratio ($P < 0.10$) which is usually regarded as a measure of biochemical age in connective tissue (Sobel *et al.*, 1958).

The mechanical properties of skin also indicated differences between the two groups of the study. The higher 'elastic stiffness' of skin in the trained versus the untrained group may, however, be explained by the thicker skin in the athletes as suggested by the chemical analyses of skin biopsies in experiment I. The possible methods for measuring skin thickness (skinfold calipers, skin biopsies etc.) were not regarded as accurate enough for use in the mechanical analyses in experiment II, especially as to the calculation of individual values for stress and strain. Therefore, the present 'elastic stiffness' values are the products of both the mechanical properties and thickness of skin. Since as age advances there is an associated rise in elastic modulus but a fall in skin thickness (Grahame, 1970), it is understandable that no significant correlation existed between age and 'elastic stiffness' in the present study.

To eliminate the influence of skin thickness, the results were calculated for 'elastic efficiency', i.e. the recovery of the energy used in the deformation of skin. Compared to the 'elastic stiffness', the efficiency values were also found to be less dependent on the creep phenomenon during the relatively slow deformation of skin. This latter variable showed a significantly better recovery of skin after deformation in the endurance athletes compared to the sedentary men. The biological basis and significance of this finding is not, however, entirely clear. It is not yet definitely known whether they are the mechanical properties of skin alone that are being measured, although Grahame (1970) came to the conclusion that *in vivo*, the circle of healthy skin behaves as a free-moving diaphragm uninhibited by underlying tissues, i.e. significant tethering does not occur. Moreover, the complexity of skin itself makes the interpretation of the results difficult. Elasticity, viscous and plastic elements are also involved in the functional properties of this type of tissue (see Viidik, 1973a), and the role of e.g. subcutaneous fat needs more examination.

Because the most abundant dry material in skin is collagen, it seems reasonable to assume that the structure and properties of collagen also largely determine the mechanical properties of skin (Fry, Harkness & Harkness, 1964). It is generally accepted that the mechanical strength of connective tissues is related to the amount of covalent crosslinks in collagen (Bailey, 1970). Thus, the increased stiffness and tensile strength which is characteristic of skin with advancing age could be explained by the increase in the number of collagen crosslinks demonstrated, e.g. in ageing rat skin (Heikkinen, Mikkonen & Kulonen, 1964). The results of the chemical analyses of collagen in the present study did not, however, show any major differences between the two groups which could explain the different mechanical properties of skin among physically active and sedentary men.

On the other hand it has also been stated that the living dermis closely resembles a gel in which elastic fibres allow stretching of the dermis by virtue of their elastic properties, but when stretched they then prevent over-extension and rupture of the collagen gel (Jarret & Matthews, 1973). It is known that long-range extensibility and rubber-like recoil are particularly characteristic of elastin (Partridge, 1973). There is also evidence that elastin has typical age-related changes in its structure and content in skin, e.g. the total amount decreases and desmosine crosslinks increase with ageing (Bartos & Ledvina, 1971). Additional work on the role of elastin and ground substance is needed to clarify the chemical basis of the mechanical properties of skin.

The absence of significant correlation between age and 'elastic efficiency' (-0.24 for the trained and -0.11 for the sedentary men) is probably due to the limited number of old people in this study. Hollingsworth *et al.* (1965) found a correlation coefficient of 0.60 between age and skin elasticity by studying the time required for a standard 'pinch' to flatten. No marked increase in the flattening time, however, occurred until after the age of 60 years. Similarly, the correlation coefficient of 0.60 between age and elastic modulus observed by Grahame (1970) was mainly derived from the changes in older age groups. To ensure the biological relevance of 'elastic efficiency', a group of twelve 71-year-old and twelve 25–31-year-old moderately active men was analysed afterwards (Suominen *et al.*, unpublished data). The values of the young men were found to be significantly higher than those of the older subjects ($35.5 \pm 3.5\%$ vs $28.5 \pm 1.5\%$, $P < 0.001$). If these results are included the correlation between chronological age and 'elastic efficiency' reached a value of -0.43 ($P < 0.001$).

Among the findings of the present study, the 'hypertrophy' and high 'elastic efficiency' of skin in the athletes might be interpreted as training effects which are in opposition to the effects of ageing. The comparison between physically active and sedentary men is, however, limited by the absence of significant correlations with age. Therefore further studies with regression analyses among subjects of wide age ranges are needed to elucidate the possible connections between habitual physical training and the biochemical and functional ageing of skin.

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REFERENCES

- BAILEY, A.J., PEACH, C.M. & FOWLER, L.J. (1970) The Biosynthesis of intermolecular crosslinks in collagen. In: *Chemistry and Molecular Biology of the Intercellular Matrix* (Ed. by E.A. Balazs), Vol. 1, p. 385. Academic Press, London and New York.
- BAKERMAN, S. (1962) Quantitative extraction of acid-soluble human skin collagen with age. *Nature*, **196**, 375.
- BARTOŠ, F. & LEDVINA, M. (1971) Age changes of cross-links in rat skin elastin. *Archiv für dermatologische Forschung*, **241**, 134.
- BOOTH, F.W. & GOULD, E.W. (1975) Effects of training and disuse on connective tissue. In: *Exercise and Sport Sciences Reviews* (Ed. by J.H. Wilmore and J.F. Keogh), p. 83. Academic Press, New York.
- CLAUSEN, B. (1962) Influence of age on connective tissue; hexosamine and hydroxyproline in human aorta, myocardium, and skin. *Laboratory Investigation*, **11**, 229.
- CLAUSEN, B. (1963) Influence of age on chondroitin sulfates and collagen of human aorta, myocardium, and skin. *Laboratory Investigation*, **12**, 538.
- DICK, J.C. (1951) The tension and resistance of stretching of human skin and other membranes with results from a series of normal and oedematous cases. *Journal of Physiology*, **112**, 102.
- FLEISCHMAJER, R., PERLISH, J.S. & BASHEY, R.I. (1972) Human dermal glycosaminoglycans and ageing. *Biochimica et Biophysica Acta*, **279**, 265.
- FRY, P., HARKNESS, M.R.L. & HARKNESS, R.D. (1964) Mechanical properties of the collagenous framework of skin in rats of different ages. *American Journal of Physiology*, **206**, 1425.

- GATT, R. & BERMAN, E.R. (1966) A rapid procedure for the estimation of amino sugars on a micro scale. *Analytical Biochemistry*, **15**, 167.
- GRAHAME, R. (1970) A method for measuring human skin elasticity *in vivo* with observations on the effects of age, sex and pregnancy. *Clinical Science*, **39**, 223.
- HEIKKINEN, E. (1968) Transformations of rat skin collagen. *Acta Physiologica Scandinavica*, **74** (Suppl.), 317.
- HEIKKINEN, E. & VUORI, I. (1972) Effect of physical activity on the metabolism of collagen in aged mice. *Acta Physiologica Scandinavica*, **84**, 543.
- HEIKKINEN, E., MIKKONEN, L. & KULONEN, E. (1964) Age factor in the maturation of collagen. Cross-links in heat-denaturated collagen in tail tendon and skin of rat. *Experimental Gerontology*, **1**, 31.
- HOLLINGSWORTH, J.W., HASHIZUME, A. & JABLON, S. (1965) Correlations between tests of aging in Hiroshima subjects—an attempt to define 'physiologic age'. *Yale Journal of Biology and Medicine*, **38**, 11.
- JARRET, A. & MATTHEWS, B.E. (1973) The physical nature of dermal collagen. *Rheumatology and Rehabilitation*, **12**, 32.
- KIISKINEN, A. & HEIKKINEN, E. (1976) Physical training and connective tissues in young mice—biochemistry of skin. *British Journal of Dermatology*, **95**, 525.
- LINDNER, J. (1972) Altern des Bindegewebes. In: *Handbuch der allgemeinen Pathologie VI/4, Altern* (Ed. by G. Holle), p. 243. Springer, Berlin.
- MINARI, O. & ZILVERSMIT, D.B. (1963) Use of KCN for stabilization of color in direct nesslerization of Kjeldahl digests. *Analytical Biochemistry*, **6**, 320.
- PARTRIDGE, S.M. (1973) Elastin biosynthesis and the influence of some extracellular factors of macromolecular organization. In: *Biology of Fibroblast* (Ed. by E. Kulonen and J. Pikkariainen), p. 13. Academic Press, London and New York.
- SOBEL, H., GABAY, S., WRIGHT, E.T., LICHTENSTEIN, I. & NELSON, N.H. (1958) The influence of age upon the hexosamine-collagen ratio of dermal biopsies from men. *Journal of Gerontology*, **13**, 128.
- SUOMINEN, H. & HEIKKINEN, E. (1975) Enzyme activities in muscle and connective tissue of M. vastus lateralis in habitually training and sedentary 33 to 77-year-old men. *European Journal of Applied Physiology*, **34**, 249.
- SUOMINEN, H., HEIKKINEN, E., PARKATTI, T., FORSBERG, S. & KIISKINEN, A. (1978) Effects of lifelong physical training on functional aging in men. *Scandinavian Journal of Social Medicine*, in press.
- UITTO, J. (1971) Collagen biosynthesis in human skin. A review with emphasis on scleroderma. *Annals of Clinical Research*, **3**, 250.
- VIIDIK, A. (1973a) Functional properties of collagenous tissues. In: *International Review of Connective Tissue Research* (Ed. by D.A. Hall and D.S. Jackson), Vol. 6, p. 127. Academic Press, New York and London.
- VIIDIK, A. (1973b) Rheology of skin with special reference to age-related parameters and their possible correlation to structure. In: *Frontiers of Matrix Biology* (Ed. by L. Robert), Vol. 1, *Ageing of Connective Tissues—Skin*, p. 157. Karger, Basel.
- WOESSNER, J.F., Jr (1961) The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Archives of Biochemistry*, **93**, 440.

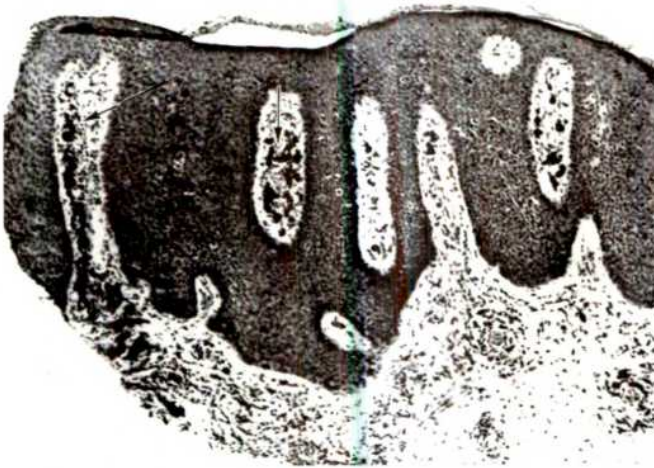


FIGURE 2. Light micrograph of epidermis from Case 1 shows the histopathological changes of carcinoma *in situ*, as well as marked pigmentation in the papillary dermis (arrows) ($\times 60$).



FIGURE 3. Epidermis from Case 3 shows changes similar to those described in Fig. 1 ($\times 250$).



FIGURE 4. Electronmicrograph of cells near the basement membrane (BM) of the epidermis. Marked plasma membrane interdigitation and abundant tonofilaments are visible. Cells on the basement membrane contain abundant melanin pigment. Case 1 ($\times 8100$).

increasing number of asymptomatic brown lesions on his penis. He had a history of having Hodgkin's disease, mixed cellularity, Stage IIB, diagnosed on September 9, 1976. He was subsequently treated with X-ray and chemotherapy agents. The penile lesions started several months after the initiation of treatment for his Hodgkin's disease. He also had a history of drug abuse and hepatitis several years prior to us seeing him. Cutaneous examination was normal except for the presence of multiple, discrete, 2–3 mm sized, flat topped, brown-coloured, pigmented papules distributed either singly or grouped as 3 or 4 conglomerate papules over the penile shaft (Fig. 1). A biopsy revealed changes of carcinoma *in*

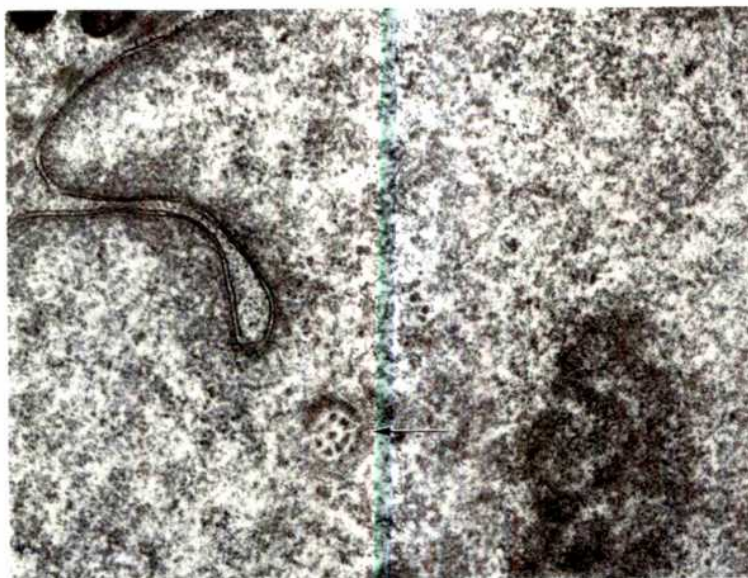


FIGURE 5. Nucleus with unusual aggregate (arrow) of approximately 50–60 nm particles. Case 1 ($\times 38,700$).

situ. The patient, on his own, medicated himself with an over the counter proprietary wart medication. He had an erythematous and crusty scaly eruption overlying the lesions that were treated symptomatically with bland topical measures when seen on 26 May, 1977. The patient refused further treatment and was lost to follow-up.

HISTOLOGY

The light microscopic findings of multiple biopsies from these three cases revealed similar histopathological features. Variable hyperkeratosis with parakeratosis, and acanthosis were seen (Figs 2 & 3). Many of the rete ridges were elongated and thickened. The epithelial cells demonstrated dysplastic changes consisting of irregularity in size, shape, and nuclear cytoplasmic ratio, involving the full thickness of the epidermis. Large hyperchromatic nuclei, mitotic figures, dyskeratotic cells and occasional multinucleated giant cells were observed. Pigment was found in the basal cell layer. The basement membrane was intact and no invasion was noted. The upper dermis demonstrated a moderate mononuclear inflammatory cell infiltrate. Aggregates of pigment-laden macrophages were seen in the papillary dermis. The pigment found in the basal cell layer and papillary dermis showed positive Fontana staining. No vacuoles or hypergranulosis was noted from the involved sites.

Electronmicroscopic examination of the histologically atypical areas revealed the following changes. The epithelial cells displayed marked interdigitation of plasma membranes, numerous desmosomes and condensed tonofilaments. Between neighbouring cells the intercellular space was not dilated as has been found in venereal warts (Lupulescu *et al.*, 1977). Many cells contained nuclei with frequent irregular invaginations and dilatation of the nuclear envelope as well as multiple nucleoli. The basal cell layer contained abundant melanin pigment (Fig. 4). The basement membrane was intact although occasionally tortuous and sometimes revealed splitting. Abnormally shaped cells with dense cytoplasm and chromatin were frequent. A few nuclei contained small particulate inclusions (Fig. 5), which were either arranged in small aggregates or scattered in the nucleoplasm.

DISCUSSION

There are recent reports in the literature of cases that resemble what we have described. Such cases have been termed carcinoma *in situ*, Bowen's disease or Bowenoid; however, most of these reports do not emphasize the unique pigmented clinical features exhibited by our three cases. Tolia *et al.* (1976) reported a 32-year-old male Caucasian patient who they described as having microscopic evidence of Bowen's disease of the penis which was successfully treated after several courses of topical 5 fluoro-uracil. Although their narration lacked complete detailed dermatological findings, a colour photograph taken of the patient's lesion was very similar to the gross appearance in our three cases.

Kopf & Bart (1977) described a 35-year old white male with multiple, erythematous to slightly violaceous polygonal papules of the glans penis that clinically resembled lichen planus, but histologically demonstrated carcinoma *in situ*. In their discussion, several other similar cases were alluded to which developed in young men and occurred on the penis. A viral association was briefly mentioned but was not established.

Lupulescu *et al.* (1977) described five cases of atypical wart-like lesions occurring over the genitalia of young individuals that histopathologically were carcinoma *in situ*. Electron microscopy failed to demonstrate viral structures in the cases that were studied. Although the clinical appearances were variable, Case 5 in their report demonstrated pigmented and erythematous papulonodular plaques of the vulva.

Pigmented papules and plaques of the genitalia and groin area are not widely known as a sign of carcinoma *in situ*. However, it has been reported in both men and women. Lloyd (1970) has described a case of squamous cell carcinoma *in situ* occurring in a 22-year-old Yemenite man. His case was characterized by extensive pigmented, jet black coloured, asymptomatic, discrete papules and plaques of the upper inner thighs. Surgical treatment was required. Lloyd reported that Hyman had encountered several similar cases. Friedrich (1972) and Skinner *et al.* (1973) described spontaneously resolving pigmented papules of the vulva associated with pregnancy in 2 teenage girls, that revealed carcinoma *in situ* changes upon biopsy. A viral aetiology was suggested in Friedrich's case. Burket (1974) described pigmented lesions showing carcinoma *in situ* changes occurring over the vulvar-perianal area in 2 patients. Both patients ultimately required extensive surgery to treat their disease.

Dysplastic changes simulating carcinoma *in situ* can also occur in patients who have condyloma acuminatum which have been treated with podophyllin (Boxer & Skinner, 1977). Such histopathological changes are transient. None of our patients had preceding treatment with podophyllin.

The pathogenesis of the PPP lesions in our patients is not known. There are reports of possible viral causation of carcinoma *in situ* in the literature (Oriel & Almeida, 1970; Oriel & Whimster, 1971; Yabe & Koyama, 1973). Our electron microscopic studies revealed a few intranuclear inclusions. Their relationship to actual viral particles or a previous viral infection can be only speculative. Some authors have stated that viral particles originally present in venereal warts are no longer demonstrable after malignant transformation has occurred (Kovi *et al.*, 1973). Thus a possible viral origin of lesions in our patients cannot be ruled out.

The distinct banal appearance and lack of symptoms of the penile papules may account for the fact that biopsy and subsequent histopathological analysis is rarely done. For this reason, such changes may be more frequent than the description from our three patients or the reported cases would imply. A biopsy is necessary to confirm their true character demonstrating the full thickness malignant intra-epithelial dysplastic alterations found in carcinoma *in situ*. The clinical picture of pigmented penile papules may suggest other benign conditions such as condyloma acuminatum, molluscum contagiosum, pigmented papilloma, pigmented naevus or seborrheic keratosis. Condyloma acuminatum and molluscum contagiosum are not usually pigmented. (In reviewing more than forty cases of venereal

TABLE 1. Summary of carcinoma *in situ* lesions of the penis

Diseases	Clinical appearance	Location	Histopathology
Penile pigmented papules	Textured flat pigmented papules	Shaft	Carcinoma <i>in situ</i>
Bowen's disease	Red scaly plaque	Shaft	Carcinoma <i>in situ</i>
Erythroplasia of Queyrat	Red moist plaque	Glans & prepuce	Carcinoma <i>in situ</i>
Leukoplakia	White plaque	Glans & prepuce	Dysplasia or carcinoma <i>in situ</i>
Bowenoid papules	Lichenoid papules	Glans	Carcinoma <i>in situ</i>

warts from our recent cases we found only two with minimal melanin pigmentation in the basal layer). The grouping and multiplicity of PPP differ from the generally solitary nature of a pigmented naevus, papilloma or keratosis. However the above benign diseases have a histopathological picture that is easily differentiated from carcinoma *in situ* found in our cases of PPP. The benign clinical appearance of PPP and the surprising carcinoma *in situ* histopathology are a combination which does not fit the classical forms of precancerous conditions affecting the penis. Penile precancerous lesions, demonstrating histological carcinoma *in situ* changes, include leukoplakia (L), erythroplasia of Queyrat (EQ), Bowen's disease (BD), and recently described Bowenoid (B) diseases (Kopf & Bart, 1977). The differential clinical and histopathological features of the L, EQ, BD, B and PPP are summarized in Table 1. PPP presents a clinical picture of small isolated and/or grouped flat-topped pigmented papules with a delicate texture located on the penile shaft. This appearance is different than the usual description found in L, EQ, BD & B. However they all share a common histopathological pattern of carcinoma *in situ*. PPP have prominent epidermal and superficial dermal pigmentation accounting for their brown clinical appearance. This pigment resembles melanin on light and electronmicroscopic study. The cause of this pigmentation is not known but post inflammatory pigmentation is a possibility.

We believe that the appearance of histopathologically documented carcinoma *in situ* of the penis can be varied. The various names applied to such lesions may mislead the clinician into looking for a stereotyped clinical picture; however, one should have a broader outlook clinically of what could be carcinoma *in situ* of the genitalia. Clinically, carcinoma *in situ* of the penis can occur in young individuals, be located on the glans penis or penile shaft and be either isolated or multifocal. The lesions or lesions may be white coloured; eroded; moist erythematous; dry erythematous; scaly; lichenoid; or pigmented papule(s), patch or plaque. The features of carcinoma *in situ* can be demonstrated only by performing a biopsy and subsequent histopathological analysis.

We are of the opinion that pigmented penile papules are a recognizable entity of the penis. The clinical picture of carcinoma *in situ* of the penis should then be broadened to include the discrete and/or grouped and conglomerate pigmented papules with a textured topography; we encourage a biopsy of such suspicious lesions. The nature and course of PPP is not known. The association with other internal malignancy, arsenic ingestion or immuno-deficiency needs further study. Some cases of carcinoma *in situ* (L, EQ, BD) of the penis may develop into invasive forms of squamous carcinoma

(Boxer & Skinner, 1977). It is not known whether PPP shows this serious potential of invasion. Therefore, cases of PPP should be followed very carefully.

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REFERENCES

- BOXER, R.J. & SKINNER, D.J. (1977) Condylomata acuminata and squamous cell carcinoma. *Urology*, **9**, 72.
- BURKET, J.M. (1974) Dark plaques in nether regions. A sign of carcinoma *in situ*. *Journal of the American Medical Association*, **230**, 439.
- FRIEDRICH, E.G. (1972) Reversible vulvar atypia. *Obstetrics and Gynecology*, **39**, 173.
- KOPF, A.W. & BART, R.S. (1977) Multiple Bowenoid papules of the penis. A new entity? *Journal of Dermatology, Surgery and Oncology*, **3**, 3.
- KOVI, J., TILLMAN, R.L. & LEE, S.M. (1973) Malignant transformation of condyloma acuminatum. *American Journal of Clinical Pathology*, **61**, 702.
- LLOYD, K. (1970) Multicentric pigmented Bowen's disease of the groin. *Archives of Dermatology*, **101**, 48.
- LUPULESCU, A., MEHREGAN, H., HOMAYOON, R., PINKUS, H. & BIRMINGHAM, D. (1977) Venereal warts vs Bowen's disease. A histologic and ultrastructural study of five cases. *Journal of the American Medical Association*, **273**, 2520.
- ORIEL, J.D. & ALMEIDA, J.D. (1970) Demonstration of virus particles in human genital warts. *British Journal of Venereal Disease*, **46**, 37.
- ORIEL, J.D. & WHIMSTER, I.W. (1971) Carcinoma *in situ* associated with virus-containing anal warts. *British Journal of Dermatology*, **84**, 71.
- SKINNER, M.S., STERNBERG, W.H., ICHIMOSE, H. & COLLINS, J. (1973) Spontaneous regression of Bowenoid atypia of the vulva. *Obstetrics and Gynecology*, **42**, 40.
- TOLIA, B.M., CASTRO, V.L., MOUDED, I.M. & NEWMAN, H.R. (1976) Bowen's disease of the penile shaft successfully treated with 5-fluoro-uracil. *Urology*, **7**, 617.
- YABE, Y. & KOYAMA, H. (1973) Virus and carcinogenesis in epidermodysplasia verruciformis. *Gann*, **64**, 167.

Allergic contact dermatitis due to sesquiterpene lactones

A COMPARATIVE STUDY OF HUMAN AND ANIMAL SENSITIVITY TO α -METHYLENE- γ -BUTYROLACTONE AND DERIVATIVES

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SUMMARY

Several compounds containing the α -methylene- γ -butyrolactone moiety have been tested on human volunteers and on guinea-pigs; the animals were experimentally sensitized by alantolactone, isoalantolactone and laurel oil. Of the two new lactones, spiro lactone was the more reactive: this was confirmed by both animal and human testing. The synthetic lactones are less reactive than natural ones. α -Methylene- γ -butyrolactone itself does not elicit cross-reactions in guinea-pigs sensitive either to alantolactone or to isoalantolactone, or in patients sensitive to sesquiterpene lactones. The α -methylene- γ -butyrolactone group is necessary for cross-reaction, but to be active, it has first to be substituted. It was also found that isoalantolactone, allegedly not allergenic, is in fact a sensitizer and cross-reacts with alantolactone. The cross-reaction between laurel and *Frullania*, found in man, also occurs in guinea-pigs. It is more evident when sesquiterpene lactone is the sensitizer and laurel used to elicit reaction.

There is abundant evidence in the literature that adverse skin reactions are caused by sesquiterpene lactones (Mitchell *et al.*, 1971a; Yoshioka, Mabry & Timmerman, 1973) contained in plants, mostly of the Compositae family. Their activity has been attributed to the presence, in the molecule, of an α -methylene- γ -butyrolactone group (Fig. 1).

Cross-sensitivity to several sesquiterpene lactones is well-documented (Mitchell & Dupuis, 1971b; Mitchell, Dupuis & Geissman, 1972), but the factors affecting the allergic activity are not known precisely. In a general approach to the understanding of the mechanism of allergic contact dermatitis,

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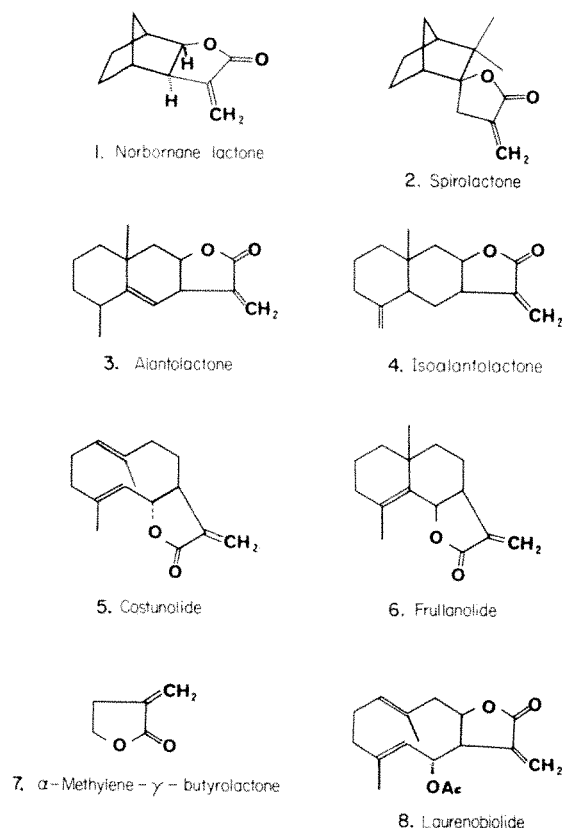


FIGURE 1.

(Schlewer, Stampf & Benezra, 1978), we started the synthesis of compounds with a rigid molecular configuration and with an α -methylene- γ -butyrolactone group. Two such lactones have been prepared (Schlewer *et al.*, 1978) **1** and **2**.

It was hoped that varying the molecular skeleton attached to the lactone moiety might lead to a better understanding of the haptene-protein conjugation allegedly necessary before the allergic reaction can occur (Gell & Benacerraf, 1961).

In addition, cross-sensitivity has been found (Asakawa *et al.*, 1974) to the lactone frullanolide **6** in a liverwort, *Frullania*, and to the essential oil of *Laurus nobilis* (used in perfumery and in medicaments such as Vegebom ointment in France). These findings were confirmed in British Columbia (Mitchell, 1975) and prompted an investigation into the nature (a sesquiterpene lactone?) of the laurel allergen.

The study reported here concerns: (a) cross-sensitivity to synthetic and natural lactones in man and (b) the induction of allergic contact dermatitis (ACD) in guinea-pigs by selected lactones (alantolactone **3**, isoalantolactone **4**) and laurel oil. Cross-sensitivity was not found in all cases and this gave some insight into the conditions necessary for lactones to elicit ACD.

MATERIAL AND METHODS

Compounds. Norbornane lactone **1** and spirolactone **2** have been synthesized and reported elsewhere (Schlewer *et al.*, 1978). Helenin, obtained from Sigma products, was separated into alanto-

lactone **3** and isoealantolactone **4**, according to the method described by Dupuis, Mitchell & Towers (1974). Costunolide **5** and laurenobiolide **8** were generous gifts from Dr H. Tada, Shionogi Research, Tokyo, Japan; frullanolide **6** was a gift from Professor Ourisson, University Louis Pasteur of Strasbourg. Laurel essential oil was obtained through the courtesy of Dr P. Teisseire, Roure-Bertrand and Justin Dupont, Grasse. The 'residue' was obtained from the essential oil by vacuum distillation (18 torr, t° 70°C): about 2/3 could be distilled off ('light fractions'), leaving 1/3 as the 'residue'. Previous studies (Foussereau, Benezra & Ourisson, 1967) have shown that the allergen is in the residue fraction. α -Methylene- γ -butyrolactone **7** was prepared, according to the method of Grieco & Pogonowski (1974), by M Jean-Pierre Corbet.

Human volunteers were from the Consultation d'allergie, Clinique Dermatologique, Hospices Civils de Strasbourg, and from the Clinique Dermatologique, Hôpital Saint-André, CHU de Bordeaux. They were patch-tested on the back, using 'neodermotests' from ROC (France): these are clover-shaped 'hypoallergenic' adhesive notches. The tests were removed and read at 48 h.

CASE REPORTS

Case 1. S., a 72-year-old retired man, was hospitalized for a generalized but predominantly facial eczema, in January, 1977. Two months later, he was discharged home. A relapse in June necessitated readmission. Patch tests revealed sensitivity to sesquiterpene lactones (as shown in Table 1) and to Vegebom, an ointment used in France for mosquito bites. The patient denied ever having used this ointment; this contains laurel oil, in which there are sesquiterpene lactones (Tada & Takeda, 1972). It is difficult to trace the aetiology in this case, for the patient has numerous Compositae plants in his garden.

Case 2. M., a female typist aged 49 years, presented in June 1977 with dermatitis on the forearms. Patch-tests revealed sensitivity to *Frullania dilatata* (+++) and *F. tamarisci* (+++). Topical corticosteroids cleared the eruption.

Case 3. C., a male chauffeur aged 32 years, was seen initially in November 1975, on account of a dry dermatitis of the face, the back of the hands and the feet. Preliminary studies showed sensitivity to potassium chromate (+++), lavender essential oil (+++), *F. dilatata*, Balsam of Peru (+) and Δ^3 -carene (++). Re-investigation in June 1977 confirmed the sensitivity to *F. dilatata*, but the patch test to *F. tamarisci* was negative.

Case 4. G. is a 59-year-old retired man with the nephrotic syndrome who attends the Nephrology service at the CHU Besançon (France). He was hospitalized three times in 1973, 1974 and 1975. He also suffers from contact dermatitis due to *Frullania*. Patch-tests revealed sensitivity to α -methylene- γ -butyrolactone derivatives (frullanolide ++, costunolide +++) and also to laurel oil.

Animal sensitization and testing. The Magnusson & Kligman (1970) maximization test was used to sensitize Hartley albino female guinea-pigs weighing between 400 and 600 g and fed with vitamin C rich granules and occasionally with lettuce and carrots. Sensitizing solutions were: 1% alantolactone, 1% isoealantolactone and 20% laurel essential oil residue in olive oil-acetone (4:1) with added Freund's complete adjuvant from Difco. To elicit reactions, 20 μ l of a solution of the allergen in olive oil-acetone (1:9) was deposited on the animal's shaved flank (epicutaneous challenge test).

Before starting sensitization, the toxicity thresholds of all the substances were checked by prelimin-

ary epicutaneous application of the solutions in various concentrations. The tests were read at 24 and 48 h (when they were maximum). Grading was from 0 to + + + as recommended by Schulz *et al.* (1977).

RESULTS

Human volunteers were patients initially sensitive to *Frullania*. The results of patch testing are recorded in Table 1. All patients reacted to frullanolide **6**, but none to α -methylene- γ -butyrolactone **7** (known to be the hapten of tulip bulbs). Although the reactions to alantolactone **3** and isoalantolactone **4** were weak, they were real and were confirmed by negative patch tests to all substances in six controls. When the two synthetic lactones **1** and **2** were tested, all patients, without exception, reacted moderately to strongly to the spiro lactone **2**. Patient **1** was also tested and found sensitive to laurel essential oil (+ + +). A preliminary conclusion from these results is that an α -methylene- γ -butyrolactone moiety is a *necessary* but not *sufficient* requirement for ACD to occur in patients and animals sensitized to sesquiterpene lactones.

TABLE 1. Patch test results

Patch tests with*†	Controls‡	1§ (M/72)	2 (F/49)	3¶ (M/32)	4** (M/38)	5†† (M/35)	6‡‡ (M/59)
Norbornane lactone 1	o	(+)	o	o	o	+	+
Spirolactone 2	o	+	+	+	+	+	+
Alantolactone 3	o	(+)	o	+	+++	+	d
Isoalantolactone 4	o	o	o	o	+++	+	+
Frullanolide 6	o	+	+	+++	+++	+	+
<i>Frullania</i>	—	—	++	++	+++	++	—
α -methylene- γ -butyrolactone 7	o	—	o	o	o	o	—

* Tested as 0.1% ethanol solution, except for *Frullania* tested as such, after wetting with water

† grading of patch-tests = (+) slight very positive reaction, + + + = strong positive reaction, o = negative, d = doubtful, — = not tested; ‡ six controls; § also tested to laurel essential oil (+ + +), Vegebom (+ + +); ¶ Also tested to potassium bichromate (+ + +) and Balsam of Peru (+);

** Also tested to potassium bichromate (+); †† Also tested to Δ^3 -carene (+);

‡‡ Also tested to laurel essential oil (+ + +), laurenobiolide (+), costunolide (+ + +).

Experiments on animals are summarized in Table 2. The average reaction for a group is calculated by adding the number of + symbols and dividing the sum by the number of animals in the group tested. The 1.7 and 2.0 average positive reaction for alantolactone **3** and isoalantolactone **4** respectively indicate that they are efficient sensitizers. We consider cross-sensitization to be clear-cut when there is a 1.0+ average reaction. Animals sensitized by alantolactone **3** cross-react to isoalantolactone **4**, spiro lactone **2**, frullanolide **6**, laurenobiolide **8**, costunolide **5** and laurel oil. Animals sensitized by isoalantolactone **4** cross-react to alantolactone **3**, spiro lactone **2**, frullanolide **6**, costunolide **5** and laurel oil, but they react only to a minor degree to laurenobiolide **8**. Animals sensitized by laurel oil cross-react only to laurenobiolide **8**, laurel oil and Vegebom.

TABLE 2. Tests on guinea-pigs

Sensitized to	Challenged with	Concentration* (%)	Epicutaneous tests†				Aver.‡	Controls§
			+++	++	+	0		
Alantolactone¶,**	Alantolactone 3	0.1	2	4	3	1	1.7	0
	Isoalantolactone 4	0.1	0	3	6	1	0.70	0
	Norbornane lactone 1	0.1	0	0	2	8	0.20	0
	Spirolactone 2	0.1	0	0	9	1	0.90	0
	Frullanolide 6	0.1	0	4	5	1	1.30	0
	Laurenobiolide 8	0.1	0	1	6	3	0.80	0
	Costunolide 5	0.1	0	0	1	9	0.10	0
	α -methylene- γ -but. 7	0.1	0	0	0	10	0	0
	Laurel ess.oil.resi.	10	0	0	9	1	0.90	0
	Laurel oil (total)	10	0	0	5	1	0.70	0
Isoalantolactone*	Isoalantolactone 4	0.1	3	1	3	0	2.0	0
	Alantolactone 3	0.1	0	3	4	0	1.4	0
	Norbornane lactone 1	0.1	0	0	2	5	0.30	0
	Spirolactone 2	0.1	0	2	4	1	1.1	0
	Frullanolide 6	0.1	0	2	4	1	1.1	0
	Laurenobiolide 8	0.1	0	0	1	6	0.14	0
	Costunolide §§5	0.1	0	0	2	0	1.0	0
	α -methylene- γ -but. 7	0.1	0	0	0	7	0	0
	Laurel ess.oil.resi.	10	0	1	6	0	1.1	0
	Laurel oil (total)	10	0	0	5	1	0.70	0
Laurel essential oil †††	Laurel ess.oil.resi.	10	1	4	3	2	1.40	0
	Alantolactone 3	0.1	0	0	4	6	0.40	0
	Isoalantolactone 4	0.1	0	0	3	7	0.30	0
	Norbornane lactone 1	0.1	0	0	0	10	0	0
	Spirolactone 2	0.1	0	0	0	10	0	0
	Frullanolide 6	0.1	0	0	2	8	0.20	0
	Laurenobiolide 8	0.1	0	2	5	3	0.90	0
	Costunolide 5	0.1	0	0	4	6	0.40	0
	α -methylene- γ -but 7	0.1	0	0	0	10	0	0
	Laurel oil (total)	10	0	0	6	2	0.80	0
	Vegebom	As such	0	0	6	4	0.60	0

* Vehicle: olive oil-acetone (1:9); † +++ = intense erythema, infiltration and exudation = 3; ++ = distinct confluent erythema and infiltration = 2; + = distinct erythema = 1; 0 = no reaction; ‡ Averaging by adding the + symbols and dividing the sum by the total number of tested animals;

§ Three controls tested with all products; ¶ Sensitized according to Magnusson and Kligman maximization test with 1% solution in an olive oil: acetone (4:1) mixture; ** One animal was desensitized after four weeks; †† Sensitized with a 20% solution as in ¶; ††† Two animals were desensitized after 4 weeks; §§ Only two animals were tested because of shortage of costunolide.

DISCUSSION

One important finding is the demonstration of cross-sensitivity between alantolactone 3 and isoalantolactone 4. Several publications have claimed that isoalantolactone is not a sensitizer (Mitchell *et al.*, 1971b; Mitchell, 1975). However, the only structural difference between the two molecules is the position of the double bond (other than the α -methylene): in alantolactone it is trisubstituted,

whereas in isoalantolactone it is exocyclic(disubstituted). Consequently the cross-sensitivity, confirmed by both human and animal testing, seems reasonable.

Another interesting result is the extreme disparity between the sensitizing power of spiro lactone **2** and that of norbornane lactone **1**. This was shown by human patch testing (all patients reacted to spiro lactone **2** but only some to norbornane lactone **1**). To our knowledge, this is the first time a spiro lactone is shown to be a significant sensitizer. Among natural spiro- α -methylene- γ -butyrolactones (Yoshioka *et al.*, 1973), none has been reported as sensitizing.

Further notable results of this study concern the difference in sensitizing power between lactones with closely related structures. Frullanolide **6** and costunolide **5** differ only in that the latter has a 10-membered ring (which can cyclize to two six-membered rings) whereas the former possesses two fused six-membered rings. The group of guinea-pigs sensitized by alantolactone **3** reacted well to frullanolide (average 1.30+) but poorly to costunolide (0.10+). The results are not so clear-cut in the isoalantolactone **4** sensitized animals (only two were tested) and so no valid conclusion can be drawn for the moment.

Because cross-sensitization occurs between *Frullania* and *Laurus nobilis* (Asakawa *et al.*, 1974), it was thought that the allergen in laurel could be a sesquiterpene lactone. Although we found that animals sensitized by laurel oil cross-reacted to laurenbiolide **8**, isolated from the roots (Tada & Takeda, 1976), their reactions to alantolactone, isoalantolactone and costunolide were weak. The cross-reaction is greater when either alantolactone or isoalantolactone is the sensitizer and laurel is used to challenge. A similar one-way type of sensitization had been described in man, notably in the case of antibiotics (Sidi, Hincky & Longueville, 1958).

CONCLUSIONS

The results presented here show that:

- (1) Human and animal responses to patch testing with sesquiterpene lactones show comparable results.
- (2) Isoalantolactone is a sensitizer, and those sensitized cross-react to alantolactone.
- (3) The allergen of laurel oil seems not necessarily to be a sesquiterpene lactone.
- (4) α -Methylene- γ -butyrolactone **7** itself does not elicit cross-reactions in subjects allergic to other lactones.
- (5) Finally, although the presence of an α -methylene- γ -butyrolactone group seems a prerequisite for cross-reactions among sesquiterpene lactones, this is not sufficient to elicit ACD.

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REFERENCES

- ASAKAWA, Y. BENEZRA, C., DUCOMBS, G., FOUSSEREAU, J., MULLER, J.C. & OURISSON, G. (1974) Cross-sensitization between *Frullania* and *Laurus Nobilis*; the allergen of laurel. *Archives of Dermatology*, **110**, 957.

- DUPUIS, G., MITCHELL, J.C. & TOWERS, G.H.N. (1974) Reaction of alantolactone, an allergenic sesquiterpene lactone, with some amino acids. Resultant loss of immunologic activity. *Canadian Journal of Biochemistry*, **52**, 575.
- FOUSSEREAU, J., BENEZRA, C. & OURISSON, G. (1967) Contact dermatitis from laurel. II. *Transactions of the St John's Hospital Dermatological Society*, **53**, 147.
- GELL, P.G.H. & BENACERRAF, B. (1961) Studies on hypersensitivity. IV. The relationship between contact and a delayed hypersensitivity. *Journal of Experimental Medicine*, **113**, 571.
- GRIECO, P.A. & POGONOWSKI, C.S. (1974) A partial synthesis of α -methylene- γ -butyrolactone. *Journal of Organic Chemistry*, **39**, 1958.
- MAGNUSSON, B. & KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea-pigs. Identification of Contact Allergens*. Charles C. Thomas, Springfield, Illinois.
- MITCHELL, J.C., ROY, A.K., DUPUIS, G. & TOWERS, G.H.N. (1971a) Allergic contact dermatitis from ragweeds (*Ambrosia* species). *Archives of Dermatology*, **104**, 73.
- MITCHELL, J.C. & DUPUIS, G. (1971b) Allergic contact dermatitis from sesquiterpenoids of the Compositae family of plants. *British Journal of Dermatology*, **84**, 139.
- MITCHELL, J.C., DUPUIS, G. & GEISSMAN, T.A. (1972) Allergic contact dermatitis from sesquiterpenoids of plants. *British Journal of Dermatology*, **87**, 235.
- MITCHELL, J.C. (1975) Biochemical basis of geographic ecology. *International Journal of Dermatology*, **14**, 239 and 301.
- SCHLEWER, G., STAMPF, J.L. & BENEZRA, C. (1978) Synthesis of, and allergic contact dermatitis to bicyclo (2.2.1)-heptyl- α -methylene- γ -butyrolactones derived from norbornene and norcamphene. *Canadian Journal of Biochemistry*, **56**, 153.
- SCHULZ, K.H., GABE, I., HAUSEN, B.M. & SIMATUPANG, M.G. (1977) The sensitizing capacity of naturally occurring quinones. Experimental studies in guinea-pigs. *Archives for Dermatological Research*, **258**, 41.
- SIDI, E., HINCKY, M. & LONGUEVILLE, R. (1958) Cross-sensitization between neomycin and streptomycin. *Journal of Investigative Dermatology*, **30**, 225.
- TADA, H. & TAKEDA, K. (1972) Structure of the sesquiterpene lactone laurenobiolide. *Chemical Communications*, 1391.
- TADA, H. & TAKEDA, K. (1976) Sesquiterpene of the lauraceae plants. Germacranolides from *Laurus nobilis* L. *Chemical Pharmaceutical Bulletin (Japan)*, **24**, 667.
- YOSHIOKA, H., MABRY, T.J. & TIMMERMAN, B.N. (1973) *Sesquiterpene Lactones*. University of Tokyo Press, Tokyo.

Circulating Sézary cells in hospitalized dermatology patients

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SUMMARY

Sézary cells in the circulation were counted daily in 14 hospitalized dermatology patients: six with contact dermatitis, five with atopic dermatitis, and three with exfoliative psoriasis. All had circulating Sézary cells, but absolute counts of Sézary cells of more than $1000/\text{mm}^3$ appeared to correlate with a severe refractory course in two patients. It is probable that low, intermittent levels of circulating Sézary cells accompany many inflammatory dermatoses, and it is reasonable that monitoring this cell population may aid in anticipating more consequential skin disease.

The Sézary cell is an atypical lymphocyte with a deeply folded or cerebriform nucleus and ample cytoplasm, often with vacuoles or pseudopods (Hoagland, 1974; Winkelmann & Caro, 1977). Small-cell variants of the Sézary cell also have been described and at times it may be difficult to distinguish these from small, normal lymphocytes on light microscopy (Lutzner *et al.*, 1973b; Zucker-Franklin, 1974). It is generally accepted that the Sézary cell is a T-lymphocyte. This is supported by studies demonstrating the lack of surface immunoglobulins detectable by immunofluorescence and of the receptor for aggregated human IgG (Flandrin & Brouet, 1974). The ability to form spontaneous rosettes with sheep erythrocytes, a reliable feature of human T-lymphocytes, is shared by the Sézary cells (Broome *et al.*, 1973; Lutzner *et al.*, 1973a). Antithymocyte globulin can cause a depression of Sézary cells (Edelson *et al.*, 1977). More specifically, recent studies indicate that Sézary cells may represent a proliferation of T-lymphocytes that act as helpers in the production of immunoglobulin (Broder *et al.*, 1976).

The question of whether the Sézary cell is a neoplastic cell, a potentially neoplastic cell, or merely a reactive cell has not been settled. The appearance of this cell in patients with non-neoplastic disease, in cultures of normal skin, and in pokeweed- or phytohaemagglutinin-stimulated lymphocyte cultures from healthy human beings points to a non-neoplastic role (Flaxman, Van Scott & Goldman, 1974; Yeckley *et al.*, 1975). This is also supported by the presence of circulating lymphokines in patients with Sézary syndrome (Umbert, Belcher & Winkelmann, 1976). However, deoxyribonucleic acid

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(DNA) measurements and chromosome investigations performed on lymphoid cells in patients with Sézary syndrome have shown the presence of chromosome markers and in some cases a high percentage of cells with tetraploid or almost tetraploid chromosome numbers (Prunieras, 1974).

Since Sézary syndrome and pre-Sézary syndrome often begin in overt forms of dermatitis, it appeared logical to us to study patients with severe generalized dermatitis for the presence of Sézary cells. We found that daily Sézary cell counts indicated a variable, cyclic presence of such cells in the blood of hospitalized patients with several diagnoses.

METHOD

During two 1-month periods in 1976 we found that 63 patients were admitted to one of two dermatology hospital services. We examined the peripheral blood smears of the patients with severe

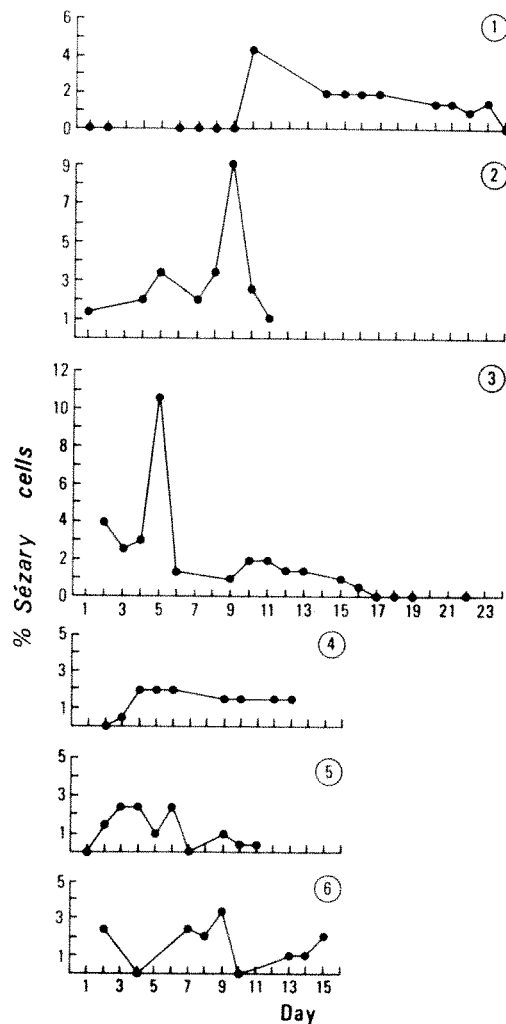


FIGURE 1. Serial percentage of Sézary cells in leukocyte count in six patients with contact dermatitis.

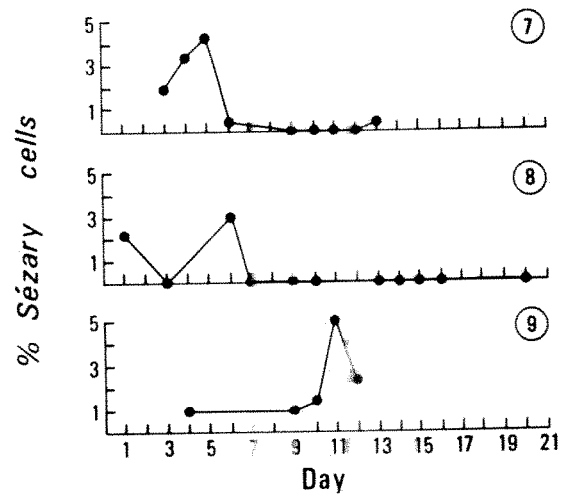


FIGURE 2. Serial percentage of Sézary cells in leukocyte count in three patients with psoriasis.

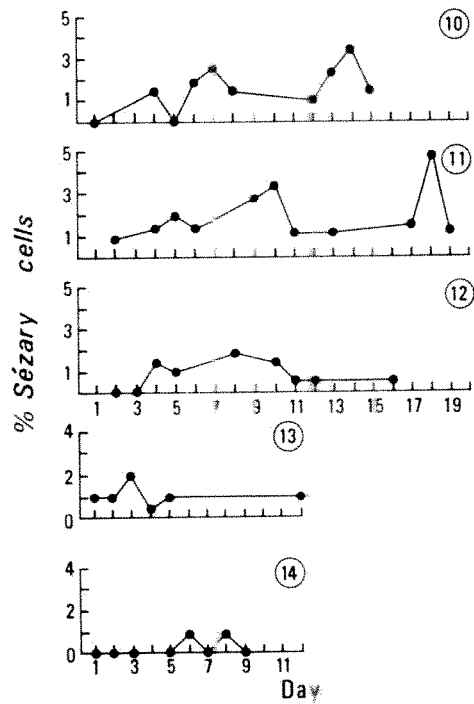


FIGURE 3. Serial percentage of Sézary cells in leukocyte count in five patients with atopic dermatitis.

generalized inflammatory dermatoses for circulating Sézary cells. One to three samples of blood were drawn from each of these patients; when Sézary cells were found in the initial counts, daily weekday counts were performed for 2 weeks or until the patient was discharged from the hospital. In two patients who had negative counts initially, serial counts of Sézary cells were made on an experimental basis because of the severe, generalized, resistant dermatitis with which they presented. The study included 14 patients: 6 with generalized contact dermatitis (Fig. 1), 3 with erythrodermic psoriasis (Fig. 2), and 5 with atopic dermatitis (Fig. 3).

Blood was drawn from the patients at 8 a.m. daily, and smears were prepared in a routine manner. Each smear was first read by a fully trained special haematology technician, counting at least 200 cells and recording Sézary cells in the differential as a percentage of white blood cells (WBC) counted. Independently, the smears were reviewed by one of the Mayo Clinic staff pathologists. He concurred with the haematology technician on each of the scores used. The specimens were sent through our Special Haematology Laboratory on a routine basis, with none of the diagnoses available to the laboratory. Serum immunoglobulins (IgG), particularly immunoglobulin E (IgE), and the erythrocyte sedimentation rate (ESR) also were measured (Table 1). Along with the total WBC and the highest and lowest percentage of Sézary cells observed, the percent of normal lymphocytes in the same count and the percent of Sézary cells as a percentage of the total lymphocytes and calculated number of Sézary cells per mm^3 of peripheral blood were determined.

The hospital treatment of these patients during the study varied according to their disease: the six patients with contact dermatitis (Cases 1-6) received tap water wet dressings over topical triamcinolone cream; two patients with psoriasis (Cases 7 and 9) were treated with the Goeckerman regimen; and the one patient with exfoliative psoriasis (Case 8) and possible paraben (Lan-E) sensitivity was treated with tap water wet dressings over paraben-free triamcinolone cream and with intravenous injections of ACTH given daily for the first 4 days of hospitalization. Four patients with atopic dermatitis (Cases 10, 11, 12, and 14) were treated initially with tap water wet dressings over triamcinolone cream and subsequently with the Goeckerman regimen; one patient (Case 13) could not tolerate tar preparations.

One patient (Case 3), a 52-year-old white woman, presented to us with a 3-year history of progressive dermatitis that seemed refractory to topical applications of steroid preparations of various strengths and brands, and she did not improve with the use of commercial lubricants or the parenteral use of steroids. Her history revealed allergy to sulphonamide drugs and a flare of her dermatitis when certain diuretic agents were used. A 3+ reaction to paraben, as well as to formaldehyde and chromate, was found on patch testing. A review of medications previously used topically revealed all to contain paraben as a preservative. The dermatitis cleared while she was hospitalized and using paraben-free topical therapy. Resolution of cyclic Sézary cythaemia as the dermatitis healed is shown in Fig. 1.

One patient (Case 4) with severe long-standing dermatitis had been treated with 2 mg of chlorambucil (Leukeran) twice daily and 25 mg of prednisone daily for 6 months before hospitalization. This treatment was continued in addition to local therapy. None of the other patients tested was receiving steroid preparations or chemotherapeutic agents orally or parenterally. Many of these patients, however, were given oral doses of antihistamines as needed for pruritus.

RESULTS

The wide fluctuation in daily Sézary cell counts is striking. Several patients demonstrated spikes of Sézary cythaemia with no associated change of symptoms or therapy. Interestingly, the patients with the highest spikes during the observation period were the most challenging patients to bring under control clinically (Cases 1, 2, 3, 7 and 11). One of our most difficult and severe cases of dermatitis

TABLE 1. Peripheral blood studies in 14 dermatology patients

[illegible]

(Case 1) failed to demonstrate Sézary cells in the peripheral smear until the second week of admission, after her dermatitis had improved clinically.

In several cases, the improvement of the patient's dermatosis clinically corresponded to a decrease in Sézary cythaemia (Cases 3, 7, 8, 9, 10 and 12). In our patients who underwent allergic patch testing during their hospital stay, no associated rise in Sézary cythaemia was noted during or subsequent to the patch testing procedure. In all of the patients having contact dermatitis, one or more positive patch tests were found.

The absolute count, or the number of Sézary cells per mm^3 of peripheral blood, appeared to correlate best with the clinical presentation. Three of our patients, to our knowledge, have done poorly since their dismissal from the hospital (Cases 1, 2 and 3) and have required continued intensive treatment or rehospitalization. These patients were also among our most difficult cases to bring under control while in the hospital. The absolute counts of Sézary cells in the circulation of these patients were among the highest recorded (423, 1143, 1380/ mm^3 , respectively).

The increased levels of IgE and the eosinophilia, which are often seen in patients with atopic dermatitis and occasionally in other forms of contact dermatitis, were noted in many of our patients, but a positive correlation with Sézary cythaemia, the severity or course of the disease, and the response to treatment were not apparent. The erythrocyte sedimentation rates, although elevated in several patients, were unrelated to Sézary cythaemia.

COMMENT

The characteristic circulating cell that was once considered to be pathognomonic for the Sézary syndrome has now been observed in patients with benign, relatively inconsequential dermatoses, as well as premalignant and malignant disorders. Lutzner, Hobbs & Horvath (1971) found the characteristic cell in the skin of 33 of 37 patients with mycosis fungoides and in the peripheral blood of 6 of 16 of these patients. The cell was present in the skin and blood of all of their patients with Sézary syndrome. The cell has been found in the skin of patients with parapsoriasis (Lutzner *et al.*, 1971) and with lymphomatoid papulosis (Schneiderman *et al.*, 1975). Flaxman, Zelazny, & Van Scott (1971) helped to establish the lack of specificity of the cell by finding the characteristic cell in the skin and the venous blood of patients with psoriasis, solar keratoses, basal cell epithelioma, lichen planus, discoid lupus erythematosus, pityriasis lichenoides et varioliformis acuta, and nonspecific vasculitis as well as with Sézary syndrome and mycosis fungoides.

In conditions other than Sézary syndrome, reports from the literature either did not comment on the quantity of Sézary cells in the blood or mentioned only low numbers. Clinical observations by one of us in 28 patients with Sézary syndrome revealed the constant features of erythroderma, oedema, pruritus, and at least 13% Sézary cells in the peripheral smear (Winkelmann, 1974). In these patients, lymphocytosis with or without leukocytosis and a highly elevated IgE were typical. In that report a Sézary cythaemia of 15% or more was suggested as a guideline in the diagnosis of Sézary syndrome.

In our present series, only 2 of the 14 patients had Sézary cells in excess of 5% (Cases 2 and 3). The same two patients were also the only patients to show maximal absolute counts in excess of 1000 Sézary cells/ mm^3 . Although these values were transient and less than adequate for the diagnosis of Sézary syndrome, we consider them significant and think that the condition of these patients should be considered pre-Sézary, being more likely to progress to Sézary syndrome or other forms of cutaneous reticulosis. In these patients, more careful observation is warranted with a more thorough search for possible antigens or aggravating factors and with strict avoidance of these substances when found. These are the patients for whom low-dose chemotherapy has been proposed (Winkelmann, 1977) and

it may be indicated if the disease cannot be controlled clinically with more conventional therapy. Long-term daily usage of low doses of chlorambucil (2-4 mg) and prednisone (20-40 mg) in such patients has produced resolution of disease which continued after all medication was discontinued (Winkelmann *et al.*, 1974).

Our patients with Sézary cells not exceeding 5% may represent a transient non-specific Sézary phenomenon demonstrating the reactive nature of the cell. We would expect the Sézary cythaemia in such patients to disappear with resolution of the acute skin problem, as it did in many of our patients. Persistent low counts and refractory dermatitis, however, may represent an early pre-Sézary state. We are continuing to study such cases.

In many types of dermatitis (O'Loughlin *et al.*, 1977) IgE has been elevated and our findings are in accord. The levels in our patients were not related to the number of circulating Sézary cells or to the course of the disease. It is of interest to recall the reported case of a patient with long-standing atopic erythroderma with grossly elevated IgE (67,000 ng/ml) and persistent low percentages of circulating Sézary cells (2%). Follow-up over a 3-year period showed continued flare of erythroderma; the IgE gradually dropped in successive determinations to 9700 ng/ml, whereas the Sézary cythaemia increased to 29.5%. These findings suggest a change in the relative activity or role of the T- and B-lymphocytes. Some of our current cases also suggest such a potential relationship.

The cyclic nature of Sézary cells in the peripheral blood when determined on a daily basis may be related to their release from skin or other lymphoid organs, production by antigen stimulation, or trapping in the skin and lymph nodes. It appears to us wise to consider constant daily levels and high levels of Sézary cells, even if intermittent, as potentially significant. Diurnal variation also may be important and should be studied. In our opinion, future absolute counts of Sézary cells should be recorded on successive days in all patients studied and reported.

We believe our observations underscore the findings of Flaxman, Zelazny & Van Scott (1971) that Sézary cells may circulate in the blood of patients with both severe and benign skin diseases. These data point to the Sézary cell as a reactive T-lymphocyte and, as such, its occurrence has some relevance to the course and the prognosis of skin disease.

We realize that from an extremely large group of patients with benign, apparently reversible, dermatitis problems often associated with identifiable antigens, a few patients will progress into the more grave diseases such as Sézary syndrome or lymphoma. Our challenge is to learn to recognize in which patients these graver diseases will evolve. In our study we have demonstrated the frequent finding of Sézary cells in the peripheral blood of patients with extensive inflammatory dermatoses of several benign causes. This is evidence that the Sézary cell *per se* is not a malignant cell but a reactive cell. The constant daily finding of a larger absolute number of these cells may be an important parameter in separating the patients with the more malignant potential from the larger group with benign disease entities. From our studies, it seems that patients with more than 1000 Sézary cells/mm³ in peripheral blood and with dermatitis that is not well controlled with conventional therapy will surely qualify for the diagnosis of pre-Sézary syndrome, thus warranting more careful study and therapy. We believe that attention to the circulating abnormal lymphocytes in refractory dermatitis patients is extremely important in the early diagnosis and treatment of more consequential disease.

REFERENCES

- BRODER, S., EDELSON, R.L., LUTZNER, M.A., NELSON, D.L., MACDERMOTT, R.P., DURM, M.E., GOLDMAN, C.K., MEADE, B.D. & WALDMANN, T.A. (1976) The Sézary syndrome: a malignant proliferation of helper T cells. *Journal of Clinical Investigation*, **58**, 1297.

- BROOME, J.D., ZUCKER-FRANKLIN, D., WEINER, M.S., BIANCO, C. & NUSSENGWEIG, V. (1973) Leukemic cells with membrane properties of thymus-derived (T) lymphocytes in a case of Sézary's syndrome: morphologic and immunologic studies. *Clinical Immunology and Immunopathology*, **1**, 319.
- EDELSON, R.L., BROWN, J.A., GROSSMAN, M.E. & HARDY, M.A. (1977) Anti-thymocyte globulin in treatment of T-cell lymphoma (letter to the editor). *Lancet*, **ii**, 249.
- FLANDRIN, G. & BROUET, J. (1974) The Sézary cell: cytologic, cytochemical, and immunologic studies. *Mayo Clinic Proceedings*, **49**, 575.
- FLAXMAN, B.A., VAN SCOTT, E.J. & GOLDMAN, L.I. (1974) Sézary-like cells in cultures of normal human skin (letter to the editor). *Archives of Dermatology*, **109**, 577.
- FLAXMAN, B.A., ZELAZNY, G. & VAN SCOTT, E.J. (1971) Nonspecificity of characteristic cells in mycosis fungoides. *Archives of Dermatology*, **104**, 141.
- HOAGLAND, H.C. (1974) Atypical lymphocytes: morphologic features. *Mayo Clinic Proceedings*, **49**, 526.
- LUTZNER, M.A., EDELSON, R.L., SMITH, R.W., SHEVACH, E.M. & GREEN, I. (1973a) Two varieties of Sézary syndrome, both bearing T-cell markers (letter to the editor). *Lancet*, **ii**, 207.
- LUTZNER, M.A., EMERIT, I., DUREPAIRE, R., FLANDRIN, G., GEMPPER, C. & PRUNIERAS, M. (1973b) Cytogenetic, cytophotometric, and ultrastructural study of large cerebriform cells of the Sézary syndrome and description of a small-cell variant. *Journal of the National Cancer Institute*, **50**, 1145.
- LUTZNER, M.A., HOBBS, J.W. & HORVATH, P. (1971) Ultrastructure of abnormal cells in Sézary syndrome, mycosis fungoides, and parapsoriasis en plaque. *Archives of Dermatology*, **103**, 375.
- O'LOUGHLIN, S., DIAZ-PEREZ, J.L., GLEICH, G.J. & WINKELMANN, R.K. (1977) Serum IgE in dermatitis and dermatosis: an analysis of 497 cases. *Archives of Dermatology*, **113**, 309.
- PRUNIERAS, M. (1974) DNA content and cytogenetics of the Sézary cell. *Mayo Clinic Proceedings*, **49**, 548.
- SCHNEIDERMAN, P., EDELSON, R., LUTZNER, M., GULLINO, M. & GREEN, I. (1975) Lymphomatoid papulosis: immunologic and ultrastructural studies (abstract). *Clinical Research*, **23**, 455.
- UMBERT, P., BELCHER, R.W. & WINKELMANN, R.K. (1976) Macrophage inhibitor factor (MIF) in cutaneous lymphoproliferative diseases. *British Journal of Dermatology*, **95**, 475.
- WINKELMANN, R.K. (1974) Clinical studies of T-cell erythroderma in the Sézary syndrome. *Mayo Clinic Proceedings*, **49**, 519.
- WINKELMANN, R.K. (1977) Nontoxic, low dose chemotherapy. *Cutis*, **19**, 309.
- WINKELMANN, R.K. & CARO, W.A. (1977) Current problems in mycosis fungoides and Sézary syndrome. *Annual Review of Medicine*, **28**, 251.
- WINKELMANN, R.K., PERRY, H.O., MULLER, S.A., SCHROETER, A.L., JORDON, R.E. & ROGERS, R.S., III (1974) The pre-Sézary erythroderma syndrome. *Mayo Clinic Proceedings*, **49**, 588.
- YECKLEY, J.A., WESTON, W.L., THORNE, E.G. & KRUEGER, G.G. (1975) Production of Sézary-like cells from normal human lymphocytes. *Archives of Dermatology*, **111**, 29.
- ZUCKER-FRANKLIN, D. (1974) Properties of the Sézary lymphoid cell: an ultrastructural analysis. *Mayo Clinic Proceedings*, **49**, 567.

Efflorescence of new warts: a sign of onset of involution in flat warts

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SUMMARY

The sudden eruption of large numbers of tiny new warts in a patient with multiple flat warts often signals the onset of involution and subsequent regression of all flat warts. We have observed 5 such cases and, in this report, describe 2 cases in which the sudden efflorescence of many new warts was used as a sign to predict accurately the onset of involution and subsequent regression of all flat warts. Correlation of the histopathological and clinical findings provides an explanation for this phenomenon.

CASE REPORT

Case 1

A 32-year-old woman with multiple flat warts on the extremities, present for 3 years, attempted to eradicate the warts by application of a solution of 10% each of salicylic, lactic and acetic acids. When seen 3 days later, multiple acutely inflamed, crusted and eroded areas were present on the thighs, legs and shoulders. The acute inflammatory process was treated with cool compresses and a steroid cream and when the inflammation subsided, a 6% salicylic acid-propylene glycol gel was prescribed for application to the warts. Four days later the patient called to report that '58 new warts' had appeared since her visit. The presence of many new flat warts was confirmed and all of the flat warts appeared inflamed, oedematous, and unusually prominent (Fig. 1). One lesion was excised for microscopic examination. When she returned in 4 weeks, all the flat warts were gone. Only a wart on the palmar aspect of the right thumb persisted.

Microscopic examination of the specimen showed a flat wart with focal accumulations of mononuclear cells around subpapillary blood vessels, exocytosis of mononuclear cells into the epidermis and the characteristic degenerative epidermal changes seen in involuting flat warts (Fig. 2).

Case 2

A 9-year-old Negro boy had flat warts on his face for a few years. In the past 2 weeks, however, many new lesions had appeared on his face and upper extremities. Alarm at the sudden outbreak of many new lesions was the reason for seeking medical attention.



FIGURE 1. Eruptive flat warts on legs of Patient 1. The lesions are erythematous, oedematous and unusually prominent.

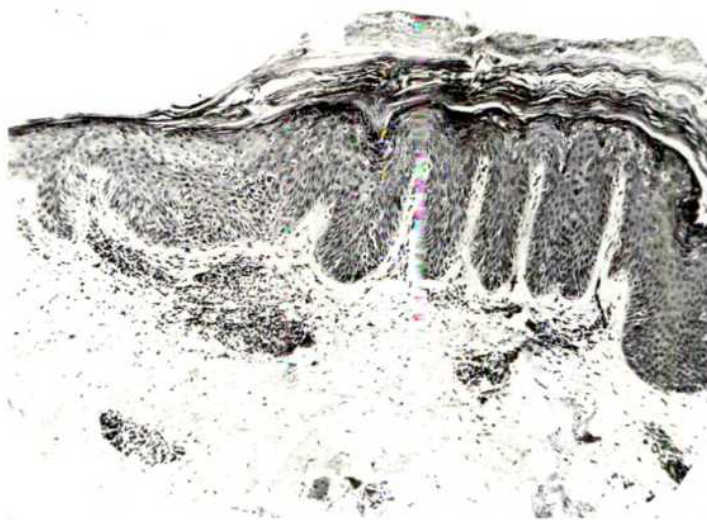


FIGURE 2. Histopathology of eruptive flat warts in Patient 1. Focal perivascular infiltration of mononuclear cells, exocytosis of mononuclear cells into the epidermis, and focal degenerative changes within the epidermis (H & E, $\times 100$).

The child's face was peppered with discrete and confluent papulonodules which appeared to be flat warts (Fig. 3). Isolated lesions were present on the forearms and dorsa of the hands and fingers. A lesion in the right preauricular area was excised for microscopic examination. When the child returned in 2 weeks, the lesions had changed dramatically; only scaling was present at the sites where the warts had been. Two weeks later, all flat warts were gone and the scaling had subsided.

Microscopic examination of the specimen excised at the initial visit showed the typical mononuclear cell infiltration of the epidermis characteristic of involuting flat warts (Fig. 4).



FIGURE 3. Discrete and confluent eruptive flat warts in Patient 2.

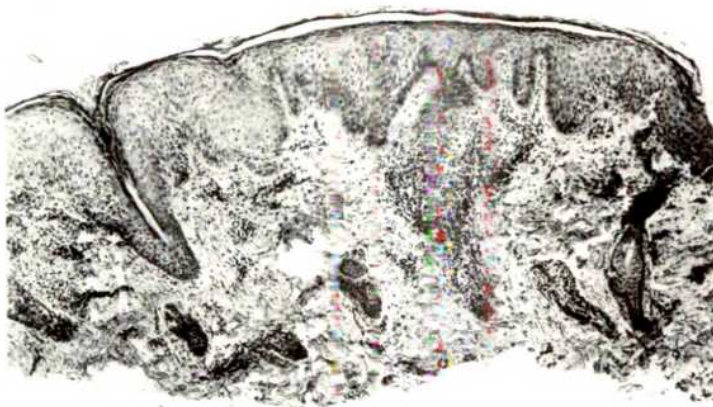


FIGURE 4. Histopathology of lesions in Patient 2. Note the mononuclear cell infiltration and spongiosis in the epidermis. All flat warts regressed two weeks after biopsy (H & E, $\times 100$).

COMMENT

The sudden eruption of large numbers of tiny new warts in patients with multiple flat warts can be utilized to recognize the onset of involution and to predict the imminent regression of all flat warts. Clinicians alert to this phenomenon can reassure the patient that this alarming development is in fact a favourable prognostic sign and that most conservative therapy is called for.

Flat warts undergoing involution are characterized histopathologically by a perivascular infiltration of mononuclear cells in the upper dermis, exocytosis of mononuclear cells into the epidermis, and degeneration and necrosis within the epidermis (Tagami *et al.*, 1974; Berman & Winkelmann, 1977). Correlation of the histopathological process with the clinical findings offers a reasonable explanation of the phenomenon. It is probable that the 'eruptive' lesions are not in fact new warts but miniscule flat warts that had not been visible previously. The mononuclear cell infiltrative process with the attendant oedema may explain the sudden dramatic appearance of 'new' lesions. The infiltrative process probably explains also the other clinical findings that suggest the onset of involution such as erythema and oedema at the base of the warts, the onset of itching of the warts, and the instability of the lesions.

REFERENCES

- BERMAN, A. & WINKELMANN, R.K. (1977) Involuting flat warts. *Archives of Dermatology*, **113**, 1219.
TAGAMI, H., OGINO, A., TAKIGAWA, M., IMAMURA, S. & OFUJI, S. (1974) Regression of plane warts following spontaneous inflammation. An histopathological study. *British Journal of Dermatology*, **90**, 147.

The release of β glucuronidase from the white cells of patients with drug rashes when incubated in autologous plasma, with and without the addition of the causal drug

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SUMMARY

In seven out of 16 patients with drug rashes, the leucocytes selectively released β glucuronidase when incubated in autologous plasma, indicating the possible presence of immune complexes. When the white cells were incubated with the suspected drug *in vitro*, they tended to react in three different ways. In four out of 16 cases additional β glucuronidase was released, perhaps due to the formation of immune complexes, in two out of the 16, β glucuronidase release was suppressed, perhaps due to antigen excess, and in two cases LDH release was decreased while β glucuronidase remained unaltered.

The addition of immune complexes to white cells in up to twentyfold antigen excess causes the release of such lysosomal enzymes as β glucuronidase (β glu) (Hawkins & Peeters, 1971). If such complexes are already present in the plasma, the incubation of leukocytes in that plasma gives rise to a similar release, increased by the addition of the causal antigen, until the state of antigen excess is reached (Watson *et al.*, 1977).

It is known that some skin rashes are caused by drug hypersensitivity and are associated with circulating complexes—the Type III reaction as classified by Coombs & Gell (1953). In order to identify the causal drug, suspected ones were added in various ratios to leukocytes incubated in autologous plasma from such patients.

MATERIALS AND METHODS

To test for complexes already present in the plasma, 20 ml blood were collected into containers each holding 20 μ g per ml pyrogen free heparin (Evans Medical). The red cells were allowed to settle for

90 min and the leukocyte rich plasma aspirated. 1.5 ml aliquots of this were diluted to 15 ml with TC 199 medium (Wellcome) at 37°C and the mixture sampled at known times for 6 h during incubation at 37°C. Cells were removed from the samples by centrifugation and the supernatants assayed after overnight storage at 4°C. LDH was assayed by the method of Bergmeyer (1963) to monitor cell death or damage and β glu by the umbelliferone method (Barrett, 1972). At each point of time the enzyme activity in the supernatant was measured in nanomoles substrate converted per hour.

Regression lines were calculated to give the rates of appearance of enzyme activity in the supernatant and these were calculated as a percentage of the total cell enzyme in the preparation as measured after Triton X lysis.

Drugs

The leukocyte rich plasma from each patient in 1.5 ml aliquots was incubated with and without the addition of the suspected drugs in various dilutions and the β glu and LDH measured in the supernatant over a period of 4–6 h.

Details of these patients are given in Table 1. Controls were selected from healthy staff members who had never been given the drug before.

RESULTS

Incubation of white cells in autologous plasma

In 38 control patients six showed evidence of selective β glu release while in all cases the LDH release was never more than 4% of the total LDH content as shown by Triton X lysis.

When the white cells from the patients with drug rashes were incubated in autologous plasma, seven out of 16 showed selective release of β glu (Fig. 1).

The addition of drugs to the leukocyte rich plasma from patients with drug rashes

Four of the 16 patients with drug rashes showed active stimulation of β glu release when their white cells were incubated in autologous plasma with the suspected drug in various dilutions (Fig. 2). These patients were O.H. on rifampicin, S.A. on ethambutol, P.B. on penicillamine, and E.A. on methyl dopa.

Two cases, however, showed suppression of β glu release when similarly treated (patients C.O. on belladonna and I.D. on isoniazid) and two showed a decrease in LDH while β glu release remained unchanged (L.E.T. and L.W.).

DISCUSSION

Constructed complexes have been shown to stimulate white cells to release the lysosomal enzyme β glu (Watson *et al.*, 1977). In these experiments monitoring with LDH showed no evidence of cell damage which might have led to the non-specific release of β glu. Plasma alone, in some of the cases reported here, both in the patients with drug rashes and in the controls, caused a high release of β glu suggesting the presence of immune complexes.

The effect of adding the suspected drug to the white cells from patients with rashes was not consistent, only four giving a further release of β glu (Fig. 2).

As Hawkins & Peeters (1971) have shown, if sufficient antigen is added to push the system into antigen excess white cells are no longer stimulated by the addition of complexes and this might have been the case in C.O. and I.D.

The diminution of LDH in two cases is surprising but has been seen in our laboratory with two

TABLE 1. Details of patients with drug rashes tested for specific enzyme release. Indications are given of non-linear β glu release similar to that seen when neutrophils are stimulated by artificially constructed complexes of bovine serum albumin-anti bovine serum albumin. Most control subjects show linear releases. LDH release rates are usually below 4% of total cell enzyme released per hour and form a control for β glu release. Except for P.B. all patients with non-linear β glu releases have total percentage enzyme releases per hour in excess of 4%. Patient P.B. was taking prednisone at assay and this is known to reduce stimulated β glu release

Patient	Illness	Drug(s) & <i>in vitro</i> concentrations tested	Non linear (stimulated) β glu released	% Total cell Enzyme released per h		Comments
				β glu	LDH	
P.B.	Rheumatoid arthritis	Penicillamine (10^{-6} - 10^{-3} M)	+	1.45*	2.27	Pemphigus * β glu release reduced by Prednisone
G.J.	Rheumatoid arthritis	Sodium aurothiomalate (0.1-100 μ g/ml)	+	37.4	3.12	Lichenified rash
K.C.	Rheumatoid arthritis	Sodium aurothiomalate (0.1-100 μ g/ml)	+	0.4	1.08	Erythematous & lichenified rash
A.C.	Rheumatoid arthritis	Indomethacin (1-1000 μ g/ml)	+	20.0	4.4	Erythematous rash
O.H.	Tuberculosis	Ritampicin (1-1000 μ g/ml)	-	1.56	2.13	Pemphigus studied 1 year after cure
Sa.	Tuberculosis	Rifampicin, isoniazid, ethambutol (1-100 μ g/ml)	-	2.30	2.37	Investigated 4 years after rash
I.D.	Tuberculosis	Rifampicin, isoniazid, ethambutol (1-100 μ g/ml)	{	8.99	2.39	Investigated at time of rash—2 estimates
L.W.	Renal tuberculosis	Rifampicin, isoniazid, paraaminosalicylic acid (1-100 μ g/ml)		10.66	3.26	14 days apart
			-	2.35	0.49	Erythematous rash
W.T.		Septtrin ($1:10^4$ - $1:10^2$ dilution)	-	1.75	1.69	Erythematous rash
A.H.		Septtrin ($1:10^4$ - $1:10^2$ dilution)	-	3.53	1.12	
L.E.T.		Barbiturates (1-10 μ g/ml)	-	2.86	0.95	Morbilloform rash
Co.		Belladonna (0.03-3.0 μ g ml $^{-1}$), phenobarbitone (1-100 μ g/ml)	-	3.3	0.123	Erythematous rash
E.A.	Hypertension	L-Methyl dopa (10^{-6} - 10^{-3} M)	+	6.7	3.22	Morbilloform rash
A.D.		Diocetyl sodium sulphosuccinate, phenylbutazone (1-1000 μ g/ml)	-	3.13	0.46	Erythematous rash
D.W.		Nitrofurantoin (1-1000 μ g/ml)	-	4.3	3.3	Eosinophilia & urticarial rash
J.D.	Epilepsy	Ethosuximide (1-1000 μ g/ml)	+	9.03	1.68	Rash

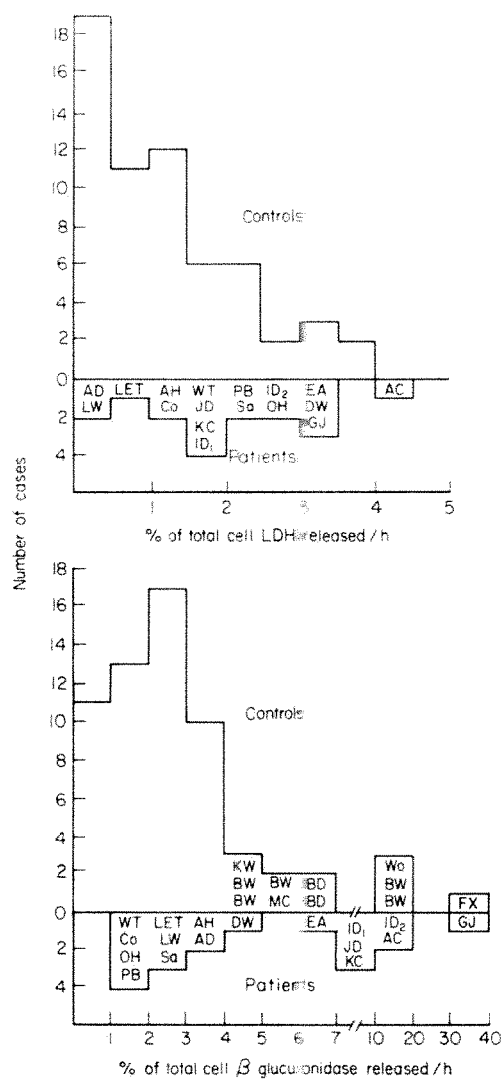


FIGURE 1. Percentage of total cell LDH and β glu released per hour from leukocytes of control subjects and patients with drug rashes. Control LDH releases were always less than 4% so that β glu releases in excess of 4% are probably stimulated and those in excess of 6% (dotted line on histogram) are certainly stimulated with non-linear release kinetics. Stimulated β glu releases were seen in some controls.

groups of cases, in lupus erythematosus when the white cells from these patients were incubated in autologous plasma and in healthy controls when very small quantities of immune complexes were added to white cells. Obviously not all rashes are due to circulating complexes but this method might be of value to identify those that are and in some cases might identify the causal drug.

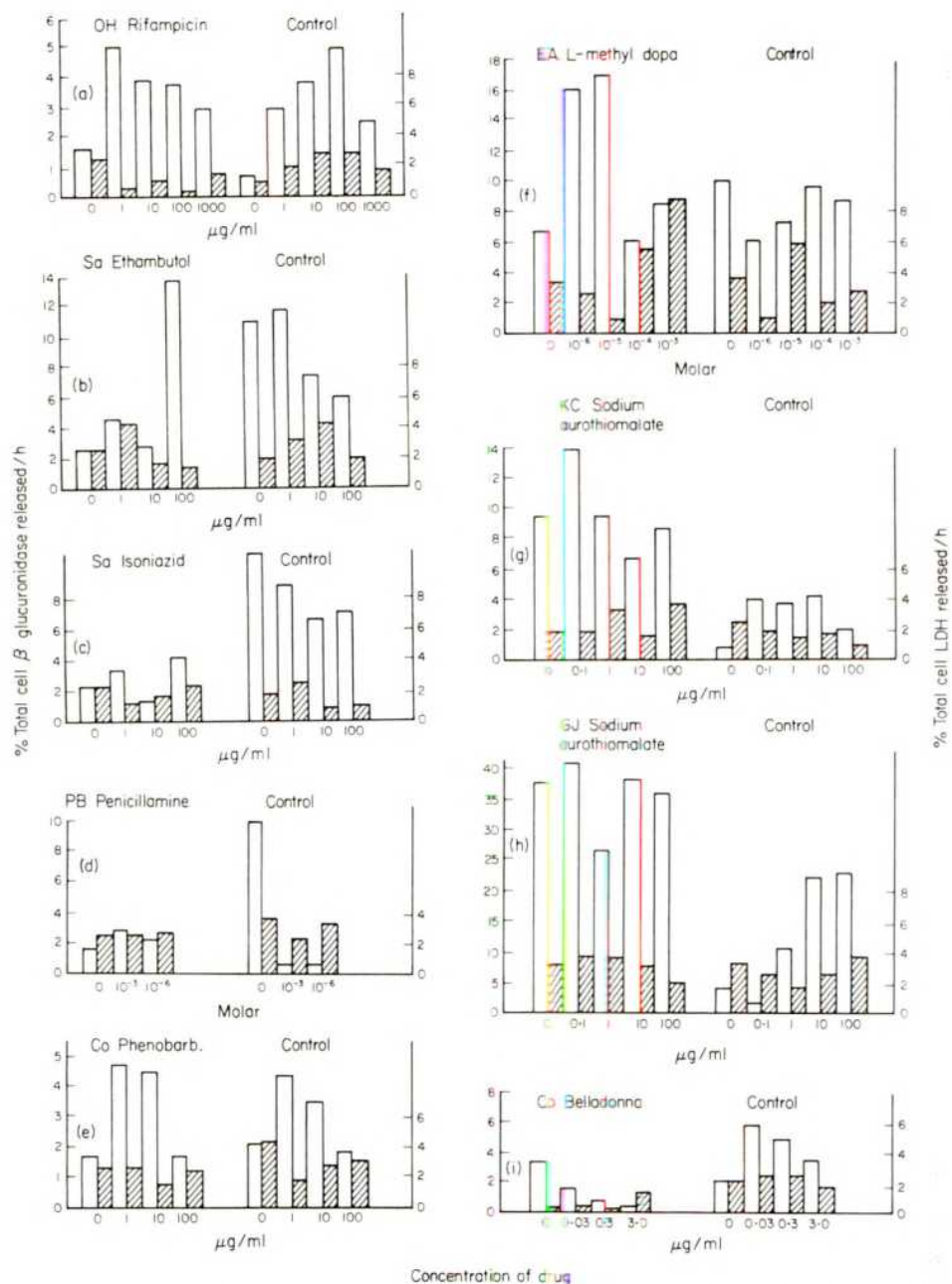


FIGURE 2. Histograms of β glu (open columns) and LDH (hatched columns) release from patients and controls in the presence of increasing concentrations of drug. Enzyme releases are expressed as the percentage of total cellular enzyme released per hour.

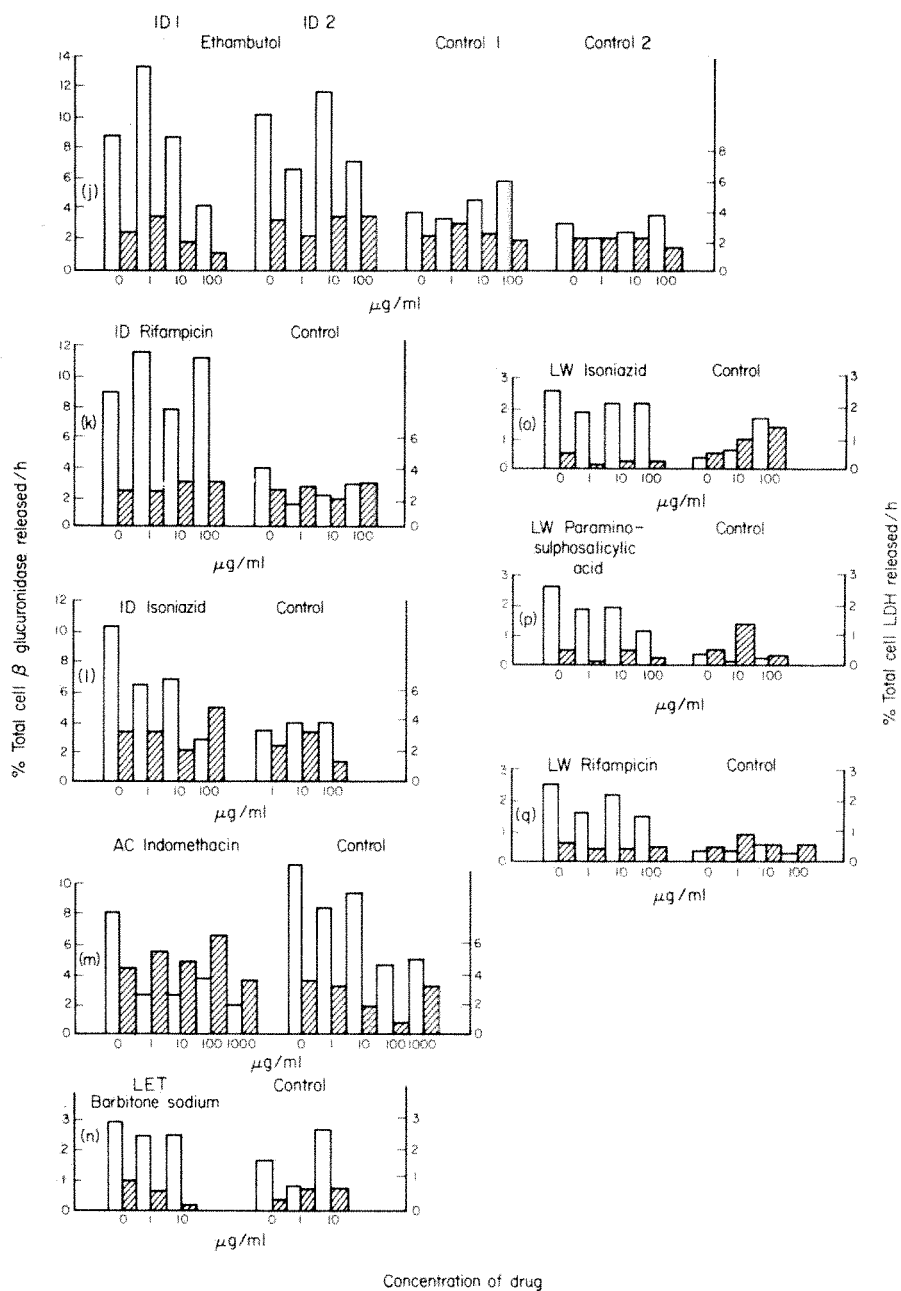
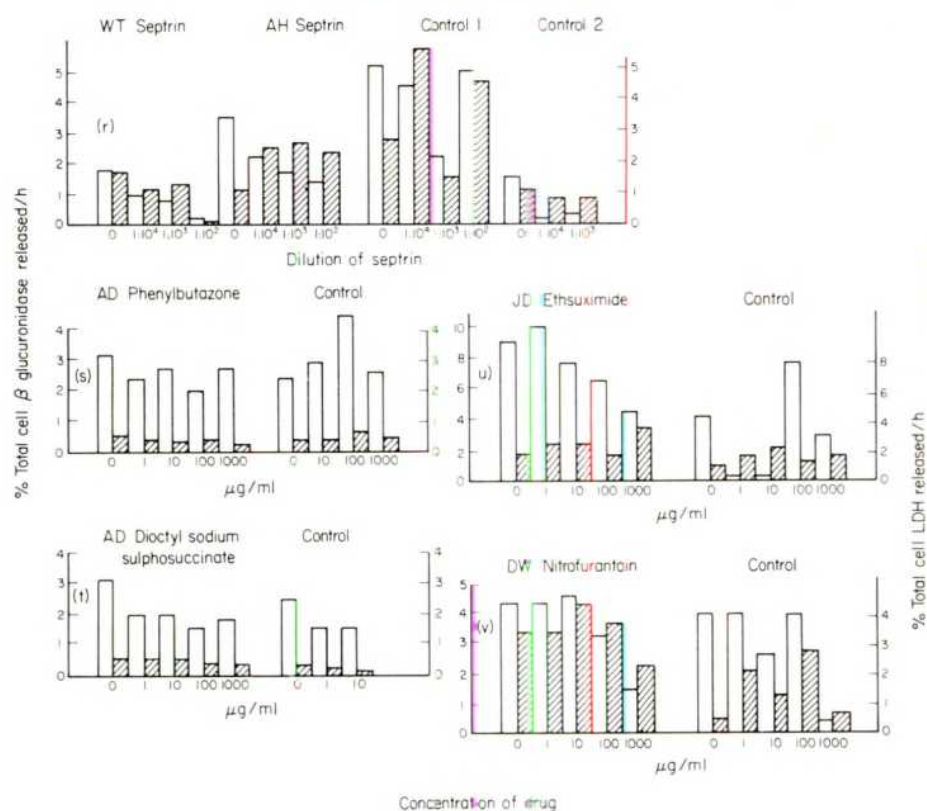


FIGURE 2 (ii).



Concentration of drug

FIGURE 2 (iii).

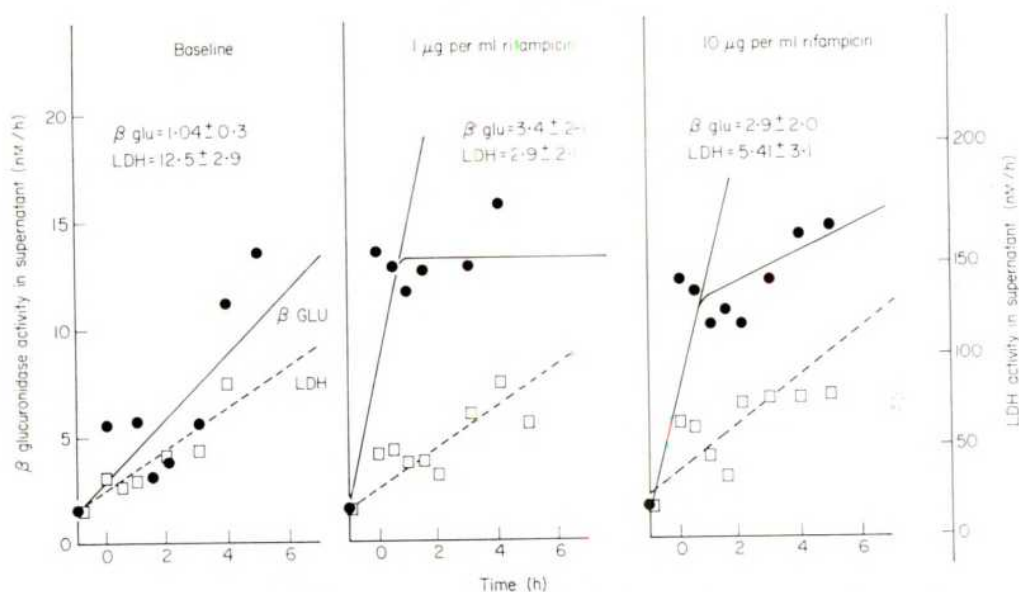


FIGURE 3. Kinetics of β glu and LDH release from leukocytes of patient O.H. spontaneously and in the presence of rifampicin. Addition of rifampicin stimulated β glu release with the establishment of non-linear profiles while LDH release was not similarly increased.

Lines drawn are not regressions but regression values for release rates as activity, in nmol substrate converted per hour, are given.

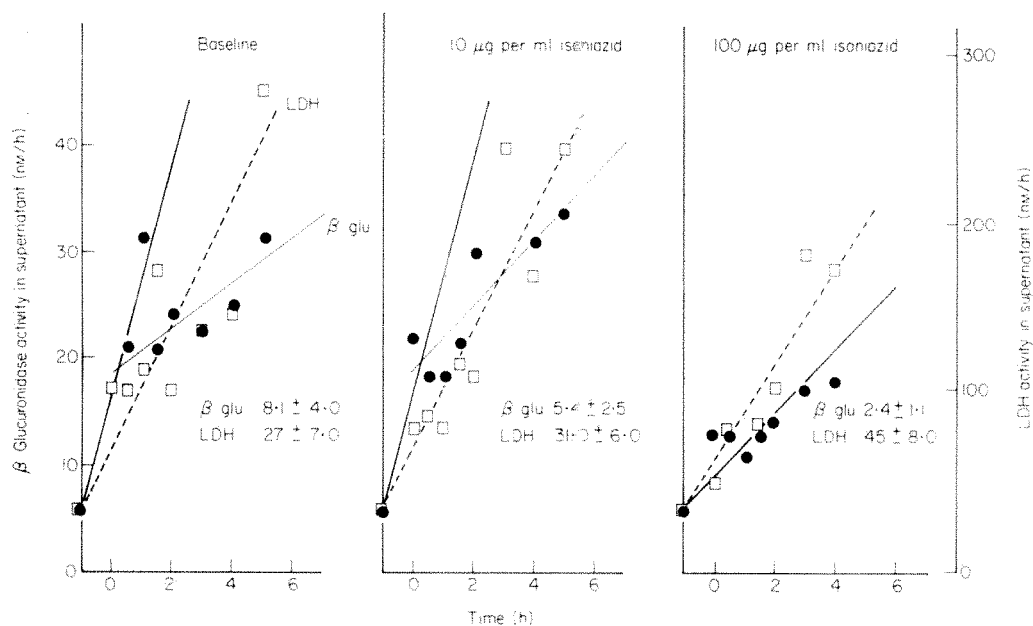


FIGURE 4. Kinetics of β glu and LDH release from leukocytes of patient I.D. spontaneously and in the presence of isoniazid. A stimulated non-linear release of β glu is halted by increasing isoniazid concentrations. Lines drawn are not regressions but regression values for release rates, as activity, in nmol substrate converted per hour, released into the supernatant per hour are presented.

ACKNOWLEDGMENTS

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REFERENCES

- BARRETT, A.J. (1972) *Lysosomes, a Laboratory Handbook* (Ed. by J.T.Dingle), p. 116. North Holland publishing Co., Amsterdam.
- BERGMEYER, H.U., BERNT, E. & HESS, B. (1963) Lactate dehydrogenase. In: *Methods of Enzymic Analysis* (Ed. by H.U.Bergmeyer), p. 736. Verlag Chemie, Academic Press, New York.
- COOMBS, R.R.A. & GELL, P.G.H. (1953) *Clinical Aspects of Immunology*. Blackwell Scientific Publications, Oxford.
- HAWKINS, D. & PEETERS, S. (1971) The response of polymorphonuclear leucocytes to immune complexes *in vitro*. *Laboratory Investigation*, **24**, 483.
- WATSON, B., GANGE, R.W., RHODES, E.L. & ROSLING, A.E. (1977) The release of β glucuronidase from leukocytes in the presence of immune complexes. *British Journal of Dermatology*, **96**, 349.

Lymphocyte transformation in patients with cobalt dermatitis

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SUMMARY

A lymphocyte transformation test with cobalt chloride was performed in 14 patients who had positive cobalt patch tests and eczema compatible with a cobalt induced contact dermatitis and in 9 controls. Eight of the patients had strongly positive patch tests, while 6 had weakly positive patch tests. Patients and controls showed equivalent responses to the mitogens, phytohaemagglutinin, pokeweed mitogen, and concanavalin A, as well as to the recall antigens, purified protein derivative and *Candida albicans*. A 1:5000 dilution in RPMI-1640 medium of a 1% aqueous solution of cobalt chloride proved optimal in distinguishing patients from controls. With this dilution, a significantly higher lymphocyte transformation was found in patients with strongly positive cobalt patch tests than in the controls. No significant difference was detected when the results for patients with weakly positive patch tests were compared with those of the controls.

The lymphocyte transformation test (LTT) has proved useful as a diagnostic tool in laboratory animals and in patients with experimentally induced contact dermatitis (Milner, 1971, 1974; Miller & Levis, 1973; Burger, Lilley & Vetto, 1974; Hinrichs & Gibbins, 1975). A specific transformation with nickel sulphate has been found by several investigators for groups of patients with nickel contact dermatitis (Svejgaard *et al.*, 1978; Hutchinson, Raffle & MacLeod, 1972; Gimenez-Camarasa *et al.*, 1975; Kim & Schöpf, 1976). A few cases of specific transformation in patients sensitized to chloramphenicol (Göring *et al.*, 1975) and chromate (Jung, 1969) have also been reported. In contrast mercury salts have been shown to act as non-specific mitogens, but not as specific antigens (Caron, Poutala & Provost, 1970).

The above reports have dealt with a limited number of patients. It has not been possible, therefore, to determine whether lymphocyte reactivity varies with the severity of the dermatitis or the intensity of the patch tests. For experimentally induced contact dermatitis in humans the degree of lymphocyte transformation appears to increase with the positivity of the patch tests (Milner, 1974; Levis, Whalen & Powell, 1975).

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In vitro diagnostic tests for allergic contact dermatitis are valuable since epicutaneous application of the specific antigen may result in a flare of the dermatitis. In some cases of widespread dermatitis, no appropriate test site can be found. The epicutaneous application of antigens may also be followed by non-specific reactivity which is indistinguishable from the allergic reaction (Hjorth & Fregert, 1972).

In the following study we have attempted to determine whether specific lymphocyte transformation can be demonstrated in patients sensitized to a cobalt salt.

PATIENTS AND METHODS

Fourteen patients who had positive cobalt patch tests ($\geq 0\%$ cobalt chloride in petrolatum) participated in the investigation. When patch-tested, all the patients had eczema compatible with cobalt contact dermatitis. The severity of the dermatitis varied greatly from patient to patient, involving minute areas of the fingers in some, and the entire body in others. There was no preponderance of extensive dermatitis in patients with strongly positive patch tests. All patients were patch tested with the European Standard Series from a few weeks to 6 months prior to the LTT. In 8 patients, the cobalt patch test was strongly positive (erythema, oedema and vesicles) after 48 and 72 h, while in 6 patients it was weakly positive (erythema, some infiltration and few papules). Eleven of the 14 patients (seven of eight with strongly positive cobalt patch tests and four of six with weakly positive cobalt patch tests) had other positive tests as well. Eight had positive nickel patch tests, and two had positive tests to chromate; four patients had 3 or more positive patch tests.

Nine persons, a few of whom had moderate acne vulgaris or psoriasis, participated as controls. None of the control persons had contact dermatitis when tested. Following the LTT, cobalt patch tests were applied to all the control persons. No positive reactions were recorded.

None of the patients or control persons had clinical or haematological evidence of infectious or other systemic disease.

The lymphocyte transformation test was performed as previously described (Svejgaard *et al.*, 1978). In brief, the following procedure was used:

Thirty ml of venous blood were drawn aseptically from each patient into bottles containing equal volumes of RPMI-1640 medium (Gibco). 480,000 units of penicillin, 0.5 g streptomycin, 50 mM hepes, 1.2 mmol glutamine, and 20,000 units of heparin were added to each litre of medium. The blood was layered on Lymphoprep (Nyc), and the lymphocytes were separated in a centrifuge at 2000 rev/min for 30 min. Then the lymphocytes were washed 3 times in 10 ml of the above medium to which 5% pooled human serum was added. For the third wash, 15% serum was added. The concentration of lymphocytes was adjusted to 10^5 per 0.5 ml by dilution with medium containing 15% serum.

Triplicate cultures were made throughout the investigation in round-bottomed culture tubes each containing 10^5 cells in 0.5 ml of medium. 50 μ l of each of the following mitogens were used: pokeweed mitogen (PWM) (Gibco) diluted 1:500; phytohaemagglutinin (PHA) (Difco), 400 μ g/ml; and concanavalin A (Con A) (Pharmacia), 500 μ g/ml. 50 μ l of a *Candida albicans* extract, 300 μ g protein/ml, and 50 μ l of purified protein derivative (PPD) (Statens Seruminstitut, Copenhagen), 10 μ g protein/ml, were used as antigens. The cobalt antigen was produced from a stock solution of 1% cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) (Specpure, Johnson Matthey) in sterile water. Dilutions of this stock solution with RPMI-1640 medium in the proportions 1:1000, 1:5000, 1:10,000, 1:50,000 and 1:100,000 were used to evaluate the dose response of three patients with strongly positive cobalt patch tests and three control persons. The remainder of the experiments were carried out with a 1:5000 dilution. Triplicate cultures with neither antigens nor mitogens served as controls. During the culture period, the tubes

were incubated at 37°C in humidified air to which 5% CO₂ was added. The cultures containing mitogen solutions were harvested after 96 h, while those containing recall antigens were harvested after 120 h. Two identical sets of cultures were made with cobalt antigen. One half of these were harvested after 120 h and the other half after 144 h. 0.05 µCi ¹⁴C-thymidine (NEN Chemicals) was added to each culture tube 24 h before harvesting with a semiautomatic harvester (Skatron). The radioactivity was measured in a liquid scintillation counter and expressed as increment counts per minute, i.e. counts of stimulated cultures (median of triplicates) minus counts of unstimulated cultures (median of triplicates).

In order to determine the toxicity of the cobalt antigen, cells from two cobalt hypersensitive patients and one control person were cultured for 96 h with PHA (400 µg/ml), and with each of the cobalt antigen dilutions used to determine dose response.

RESULTS

All the patients and controls showed response to the mitogens and recall antigens. There was no statistically significant difference between the responses of patients and controls or between the responses of patients with strongly positive cobalt patch tests and those with weakly positive patch tests.

The PHA response was moderately inhibited when a 1:1000 dilution of cobalt antigen, in addition to PHA, was added to the cultures from both cobalt sensitive patients and controls. There was no inhibition when more dilute solutions of cobalt antigen were used.

In the experiments in which serial dilutions of the cobalt antigen were used, the most distinct difference between the responses of cobalt sensitive patients and controls was seen when the 1:5000 dilution was used (Figs 1 and 2). This was true both for the harvest after 120 h and after 144 h.

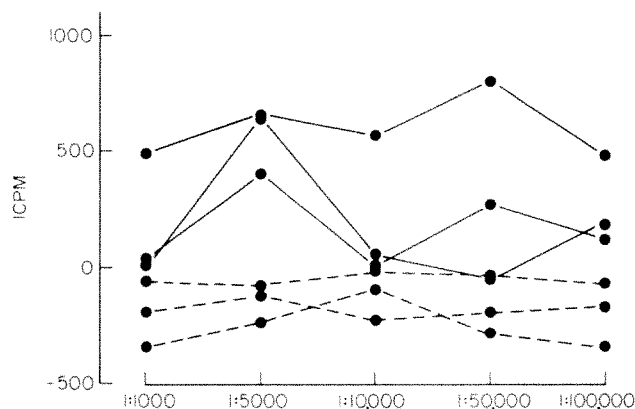


FIGURE 1. Increment counts per minute (ICPM) (for explanation see text) for 3 patients with strongly positive cobalt patch tests and 3 controls using various dilutions of the cobalt antigen and a culture period of 120 h.

When the 14 cobalt sensitive patients and 9 control persons were tested with a 1:5000 cobalt antigen dilution, patients who had strongly positive cobalt patch tests showed higher increment counts per minute than those with weakly positive tests or the control persons (Fig. 3). The difference between the responses of highly cobalt sensitive patients and the controls was statistically significant ($P < 0.01$, Mann-Whitney test) after both the 120 and the 144 h culture period. There was no statistically significant difference between the reactivity of the patients with weakly positive patch tests and the controls.

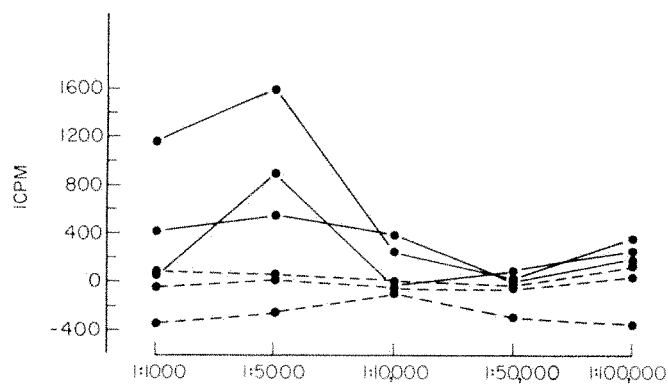


FIGURE 2. Increment counts per min (ICPM) (for explanation see text) for the 3 patients and the controls depicted in Fig. 1 after a culture period of 144 h.

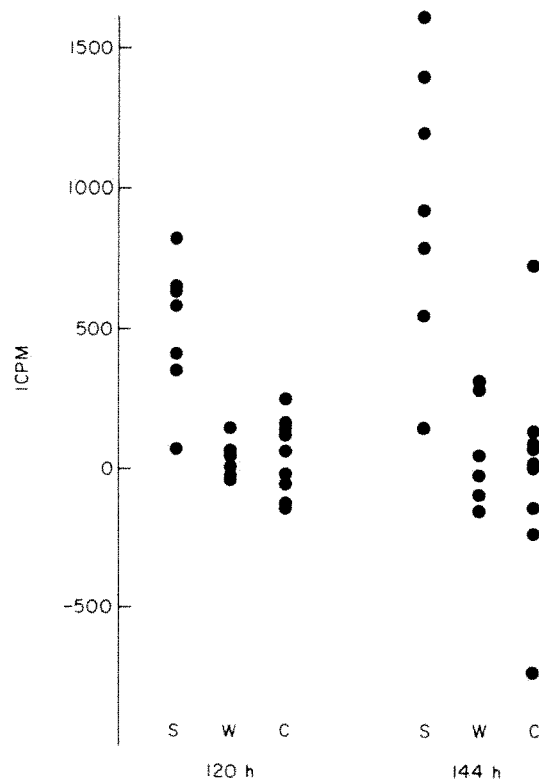


FIGURE 3. Increment counts per min (ICPM) (for explanation see text) for 7 patients with strongly positive cobalt patch tests (S), 6 with weakly positive cobalt patch tests (W) and 9 controls (C) after 120 and 144 h of culture using a 1:5000 dilution of the cobalt antigen.

DISCUSSION

The results presented here indicate that it is possible, using cobalt chloride as the antigen, to detect specific lymphocyte transformation in cobalt hypersensitive patients with strongly positive cobalt patch tests. However, the results are less encouraging than those obtained in a similar study of nickel sensitive patients (Svejgaard *et al.*, *in press*). In the latter study, specific lymphocyte transformation was detected in a group of nickel sensitive patients regardless of the degree of sensitization. This difference could be due to the fact that epicutaneous nickel contact is inevitable and that nickel ordinarily is ingested in greater amounts than cobalt (Dencker, Fristedt & Övrum, 1971), thus maintaining lymphocyte stimulation in nickel sensitive patients. An oral challenge with the antigen prior to the LTT may increase lymphocyte reactivity to cobalt. This method has been successful for the increase of lymphocyte reactivity to nickel in nickel sensitive patients (Veien *et al.*, unpublished observation).

The distribution of sensitized cells in the peripheral blood does not necessarily reflect the distribution of these cells in the body. It is not surprising, therefore, that no correlation was found between the severity of the dermatitis and the LTT reactivity. This is particularly true as the tests were made independently of periods of eruption and flare of the dermatitis. It is possible that repeated examinations of a large group of patients with carefully registered clinical conditions would show a correlation between the result of the LTT and the clinical condition.

High lymphocyte reactivity was seen in one control person after 144 h (Fig. 3). This person had never had eczema, and a cobalt patch test was negative. The finding may represent technical difficulties but could also be an indication of subclinical cobalt sensitization. A similar observation was made when aggravation of an eczema occurred following oral challenge with metal salts in patients with negative patch tests to the same metal salts (Veien & Kaaber, 1976). The results of the current study indicate that it is necessary to examine a larger number of cobalt sensitive persons and more controls in order to determine fully the specificity and sensitivity of LTT in the diagnosis of cobalt hypersensitivity.

In this study cobalt chloride was added to the cultures as the antigen. In most previous studies of leukocyte function in patients with contact dermatitis, the hapten has been coupled to serum proteins, lymphocytes, or erythrocytes in order to produce a complete antigen (Thulin & Zachariae, 1972; Miller & Levis, 1973). Similar coupling of cobalt might increase the reactivity in the LTT, although it is likely that the metal salt couples to cells or proteins in the culture tubes.

It is concluded that even though it is possible to detect specific transformation in some cobalt sensitive patients, the clinical value of the test is limited. It may prove useful when examining patients with simultaneously positive nickel and cobalt patch tests. For these patients clinical examination alone is not always sufficient to determine which of the metals is responsible for the maintenance of the dermatitis. The maintenance of LTT response appears to be dependent on repeated stimulation by the antigen (Miller & Levis, 1973). Therefore, a distinction between nickel and cobalt should be possible, if differences in LTT response to the two metals could be determined.

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REFERENCES

- BURGER, D.R., LILLEY, D.P. & VETTO, R.M. (1974) Lymphocyte suppression associated with alpha globulin changes in cellular immunity. *Cellular Immunology*, **10**, 432.

- CARON, G.A., POUTALA, S. & PROVOST, T.T. (1970) Lymphocyte transformation induced by inorganic and organic mercury. *International Archives of Allergy*, **37**, 76.
- DENCKER, I., FRISTEDT, B. & ÖVRUM, P. (1971) Järn, koppar, krom, nickel, bly och kobolt i föda. *Läkartidningen*, **68**, 4027.
- GIMENEZ-CAMARASA, J.M., GARCIA-CALDERON, P., ASENSIO, J. & de MORAGAS, J.M. (1975) Lymphocyte transformation test in allergic contact nickel dermatitis. *British Journal of Dermatology*, **92**, 9.
- GÖRING, H.D., RAITH, L., SCHUBERT, H. & SCHWALM, I. (1975) Nachweis einer Chloramphenikol-Allergie im Lymphozyten-transformationstest. *Dermatologische Monatschrift*, **161**, 612.
- HINRICHS, D.J. & GIBBINS, B.L. (1975) The *in vitro* detection of antigen sensitivity in contact dermatitis by lymphocyte transformation. *Cellular Immunology*, **18**, 343.
- HJORTH, N. & FREGERT, S. (1972) Contact dermatitis. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 1, p. 238, Blackwell Scientific Publications, Oxford.
- HUTCHINSON, F., RAFFLE, E.J. & MACLEOD, T.M. (1972) The specificity of lymphocyte transformation *in vitro* by nickel salts in nickel sensitive subjects. *Journal of Investigative Dermatology*, **58**, 362.
- JUNG, E.G. (1969) Bedeutung der Lymphoblastentransformation beim allergischen Kontaktekzem. *Therapeutische Umschau*, **26**, 94.
- KIM, C.W. & SCHÖPF, E. (1976) A comparative study of nickel hypersensitivity by the lymphocyte transformation test in atopic and non-atopic dermatitis. *Archives for Dermatological Research*, **257**, 57.
- LEVIS, W.R., WHALEN, J.J. & POWELL, J.A. (1975) Studies on the contact sensitization of man with simple chemicals. III. Quantitative relationships between specific lymphocyte transformation, skin sensitivity, and lymphokine activity in response to dinitrochlorobenzene. *Journal of Investigative Dermatology*, **64**, 100.
- MILLER, A.E., Jr & LEVIS, W.R. (1973) Studies on the contact sensitization of man with simple chemicals. I. Specific lymphocyte transformation in response to dinitrochlorobenzene sensitization. *Journal of Investigative Dermatology*, **61**, 261.
- MILNER, J.E. (1971) *In vitro* lymphocyte responses in contact hypersensitivity. II. *Journal of Investigative Dermatology*, **56**, 349.
- MILNER, J.E. (1974) *In vitro* lymphocyte responses in contact hypersensitivity IV. *Journal of Investigative Dermatology*, **62**, 591.
- SVEJGAARD, E., MORLING, N., SVEJGAARD, A. & VEIEN, N.K. (1978) Lymphocyte transformation induced by nickel sulphate. An *in vitro* study of subjects with and without a positive nickel patch test. *Acta dermatovenereologica*, *in press*.
- THULIN, H. & ZACHARIAE, H. (1972) The leucocyte migration test in chromium hypersensitivity. *Journal of Investigative Dermatology*, **58**, 55.
- VEIEN, N.K. & KAABER, K. (1976) Latent metal hypersensitivity among patients with pompholyx. *Contact Dermatitis*, **2**, 361.
- VEIEN, N.K., SVEJGAARD, E. & MENNÉ, T., Unpublished observation.

The effect of topical isoprenaline on psoriatic skin

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SUMMARY

Twelve patients with psoriasis were treated by the topical application of 0.1% isoprenaline sulphate ointment for 10 days. Topical white vaseline was used as a control in three patients with psoriasis. The mean glycogen level was found to be decreased significantly (from $120 \pm \text{s.d. } 40$ to $58 \pm \text{s.d. } 26$ mg/100 g of wet weight of involved skin) after topical application of 0.1 isoprenaline sulphate and the scaling also disappeared. Topical application of white vaseline did not produce any significant change in glycogen levels or scaling. The changes after isoprenaline application may have been due to an increase in the ratio of cyclic AMP/cyclic GMP which inhibited cell turnover and increased glycogenolysis.

Voorhees *et al.* (1971) postulated that the epinephrine-cyclic AMP cascade may control the epidermal cell cycle and glycogen metabolism. These observations were made on the basis of studies *in vitro* with dibutyl cyclic AMP and isoprenaline on the epidermis. Cyclic AMP levels were found to be decreased in psoriatic skin compared to uninvolved skin by Voorhees *et al.* (1972), but Yoshikawa *et al.* (1975) reported no significant fall in cyclic AMP levels in involved psoriatic skin compared to uninvolved skin.

Voorhees *et al.* (1975) have since suggested that the ratio of the two cyclic nucleotides CAMP and CGMP may control skin glycogen metabolism. The ratio of cyclic AMP/cyclic GMP was found to be decreased in psoriatic skin, and this was presumed to be the cause of increased cellular proliferation and decreased glycogenolysis in psoriatic skin.

In this study an endeavour was made to study *in vivo* the influence of the β -adrenergic agonist isoprenaline on the glycogen content of psoriatic skin.

MATERIALS AND METHODS

Fifteen patients with psoriasis were admitted to a Dermatology Unit. A skin biopsy (5×5 mm) was taken for glycogen estimation (Seifter *et al.*, 1950) after noting the features of the psoriatic patches. A

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group of 12 patients were treated with daily topical application to the lesions of 0.1% isoprenaline sulphate in white vaseline. One patient in this group was first tested with topical application of 0.2% isoprenaline for a short time but as erythema and mild exfoliation of skin developed this was withdrawn and 0.1% isoprenaline was substituted. The other group of 3 patients with mild lesions were treated with plain vaseline.

After 10 days of topical application of the ointment, the morphological features of the psoriatic patches were noted and a second skin biopsy was taken for glycogen estimation.

Glycogen content was also measured in the skin of 5 normal subjects.

RESULTS

The mean glycogen content of skin in the five normal subjects was found to be $35 \pm \text{s.d. } 6.9$ mg/100 g of wet weight of the tissue. The mean glycogen content of the involved skin in fifteen psoriatic patients was found to be $108.6 \pm \text{s.d. } 43.7$ mg/100 g of wet weight of tissue and this increase was statistically significant ($P < 0.01$).

Topical application of 0.1% isoprenaline sulphate ointment in 12 psoriatic patients for 10 days caused a fall in the levels of mean glycogen content from $120.2 \pm \text{s.d. } 40.3$ mg/100 g of wet weight of tissue to $58.2 \pm \text{s.d. } 26.9$ mg/100 mg of wet weight of tissue ($P < 0.01$). By contrast, the topical application of plain vaseline to three psoriatic patients did not alter the glycogen content of skin. The mean glycogen content was found to be $62 \pm \text{s.d. } 15.3$ mg/100 g of wet weight of tissue before and $61.6 \pm \text{s.d. } 20$ mg/100 gm of wet weight of tissue after topical application of white vaseline for 10 days.

The patients treated with topical isoprenaline showed the disappearance of scales (Fig. 1a & 1b). In the patients treated with vaseline, the skin showed decreased scaling during the treatment period but the scales re-appeared after the withdrawal of vaseline.

Of the 12 patients treated with 0.1% isoprenaline sulphate ointment, 3 patients returned with a relapse after a period of 2 months, but the other 9 continued in remission for up to 6 months.

DISCUSSION

The results of this study have shown a three-fold increase in glycogen content of psoriatic skin when compared to the glycogen content of normal skin. These observations are in agreement with the findings of Halprin *et al.* (1965, 1973).

Voorhees *et al.* (1975) have postulated that the two cyclic nucleotides CAMP and CGMP appear to control skin metabolism. The ratio of CAMP/CGMP was found to be decreased in psoriatic skin and this phenomenon was presumed to be the cause of the increased glycogen content and cellular proliferation in psoriasis. This hypothesis was also supported by the observation that cyclic AMP formation is reduced by the use of β -adrenergic blocking agents, antimalarials, lithium and iodides (Voorhees *et al.*, 1975). Mui *et al.* (1975) and Adachi *et al.* (1975) suggested that each drug acts independently on the epidermal adenylyl cyclase system.

In the present study the topical application of 0.1% isoprenaline sulphate in white vaseline was found to decrease significantly the glycogen content and scaliness of psoriatic skin. Application of white vaseline alone did not alter the glycogen content.

This study has shown that in addition to reducing glycogen content, topical isoprenaline can cause remission of psoriasis. Isoprenaline *in vitro* stimulates adenylyl cyclase and cyclic AMP (Voorhees *et al.*, 1971) and this present study shows that isoprenaline *in vivo* in psoriatic patients may decrease cell turnover by increasing the CAMP/CGMP ratio.

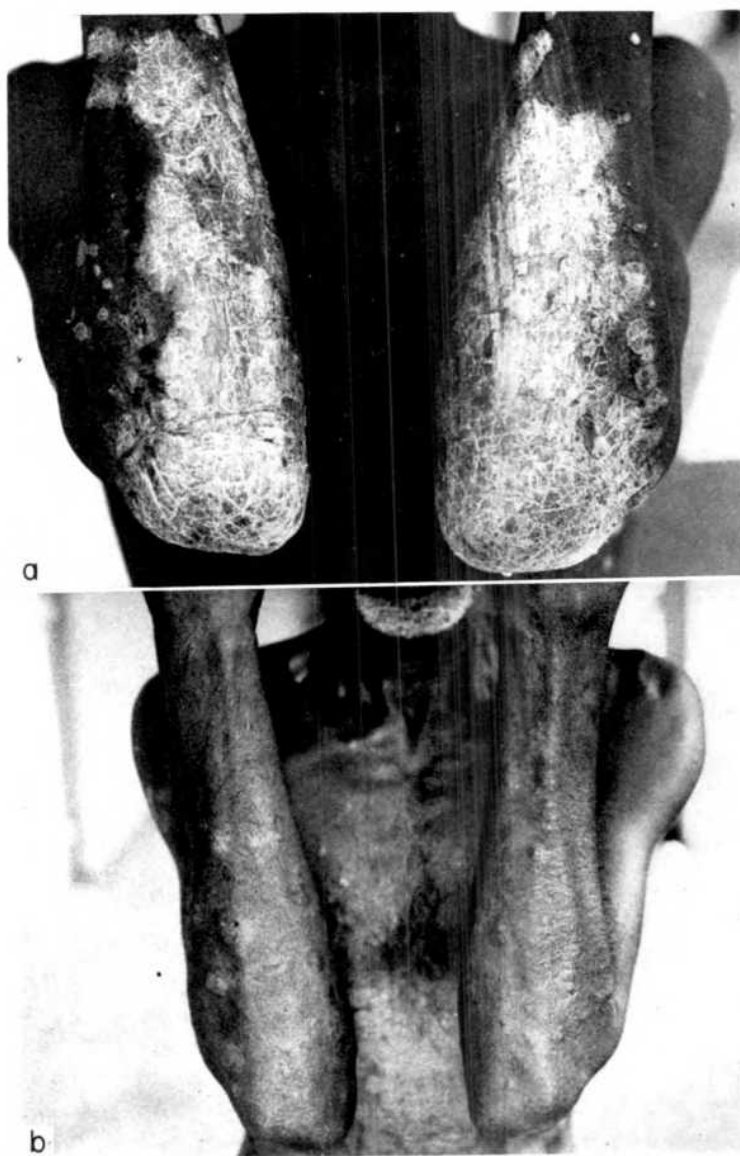


FIGURE 1. Psoriasis (a) before and (b) after application of 0.1% isoprenaline for 10 days.

ACKNOWLEDGMENTS

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REFERENCES

- ADACHI, K., YOSHIKAWA, K., HALPRIN, K.M. & LEVINE, V. (1975) Prostaglandins and cyclic AMP in epidermis. *British Journal of Dermatology*, **92**, 381.

- HALPRIN, K.M. & OHKAWARA, A. (1965) Synthesis of glycogen in psoriatic skin. *Journal of Investigative Dermatology*, **45**, 391.
- HALPRIN, K.M. & OHKAWARA, A. (1966) Carbohydrate metabolism—an enzymatic study. *Journal of Investigative Dermatology*, **46**, 51.
- HALPRIN, K.M. & OHKAWARA, A. (1973) Synthesis of glycogen in psoriatic lesion. *Archives of Dermatology*, **107**, 706.
- MEI MEI MUL., HSIA, S.L. & HALPRIN, K.M. (1975) Further studies on adenylcyclase in psoriasis. *British Journal of Dermatology*, **92**, 255.
- SEIFTER, S., DAYTON, S., NOVAC, B. & MUNTWYLER, E. (1950) The estimation of glycogen with anthrone reagent. *Archives of Biochemistry*, **25**, 191.
- VOORHEES, J.J. & DUEL, E.A. (1971) Psoriasis as a possible defect of adenyl cyclase—cyclic AMP cascade—a defective chalone mechanism. *Archives of Dermatology*, **104**, 352.
- VOORHEES, J.J., DUEL, E.A. & BASS, L.J. (1972) Decreased cyclic AMP in the dermis of lesion of psoriasis. *Archives of Dermatology*, **105**, 695.
- VOORHEES, J.J., MARCELO, C.L. & DUEL, E.A. (1975) Cyclic AMP, cyclic GMP and glucocorticoids as potential metabolic regulators of epidermal proliferation and differentiation. *Journal of Investigative Dermatology*, **65**, 179.
- YOSHIKAWA, K., ADACHI, K., HALPRIN, K.M. & LENINE, V. (1975) Is the cyclic AMP in psoriatic epidermis low? *British Journal of Dermatology*, **93**, 253.

Role of ultraviolet light and UV DNA in the induction of skin lesions in experimental animals

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SUMMARY

In vivo and *in vitro* animal experiments have been performed to clarify the role of ultraviolet light denatured DNA (UV DNA) and ultraviolet light (UVL) in the pathogenesis of the dermal lesions of human SLE. Rabbits immunized with UV DNA show deposition of immunoglobulin at the dermal-epidermal junction following exposure to UVL. We have also shown that UV DNA appears concomitantly with the antibody at the dermal-epidermal junction subsequent to the UVL exposure. Both n DNA and UV DNA have been shown to bind to the dermal-epidermal junction *in vitro* which could result in the persistence of these antigens at this site. These studies lend further support to the hypothesis that the release of UV DNA and its subsequent deposition at the dermal-epidermal junction may result in binding of both immunoglobulin and complement, leading to the development of histological lesions of SLE.

It is not clear what role ultraviolet light plays in the pathogenesis of systemic lupus erythematosus (SLE). Clinically, it is recognized that exposure to ultraviolet light may either induce SLE or, in those patients where the disease has already been diagnosed, may exacerbate it (Dubois, 1974). The fact that dermal DNA can be denatured by exposure to ultraviolet light producing UV DNA *in vivo* (Tan & Stoughton, 1969) and that this substance is highly immunogenic (Levine *et al.*, 1966) has supported the suggestion that ultraviolet light may be one pathogenic factor in SLE. Previous studies have shown that patients with SLE have antibodies to UV DNA and that these may cross react with native DNA (Davis, Russell & Percy, 1976). Patients who developed photosensitivity tended to have antibodies to UV DNA, and the titres were higher than those seen in patients who were not photosensitive. There was, however, no strong correlation but antibodies to UV DNA have not been detected in patients suffering from other forms of photosensitive skin eruption (Davis, 1977). Experimentally it has been shown in mice that whole body exposure to UV light not only induces UV DNA *in vivo* but, in animals presensitized to UV DNA, immunoglobulin and complement deposits can be seen at the dermal-epidermal junction (Natali & Tan, 1973). These changes are

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histologically similar to those seen in SLE. The presumed antigen was not identified although UV DNA released from epidermal cell nuclei would seem to be the most likely possibility. The aim of this particular study was to clarify the role of UV DNA in the dermal histological lesion induced by UV irradiation and, in addition, to examine the *in vitro* binding characteristics of both UV and native DNA to the basement membrane at the dermal-epidermal junction.

MATERIALS

Antibodies to UV DNA in serum from patients with SLE and from immunized rabbits were detected by both immunofluorescence and radioimmunoassay techniques as previously described (Davis, Russell & Percy, 1976; Tan, 1968). Twelve female New Zealand white rabbits were injected on 10 occasions with photo-denatured DNA prepared in a concentration of 1 mg/ml/rabbit/14 days. In our radioimmunoassay system UV DNA binding was in the range of 6–12% preimmunization. Maximum levels of UV DNA binding were detected after the eighth injection with a range of 13–56%. Sera from all rabbits were negative for antibodies to UV DNA using an immunofluorescence technique prior to immunization and strongly positive after the sixth injection. Rabbits were randomly divided into two groups, a control group and a group that were exposed to ultraviolet light for 1 h every day. Histological studies were performed on punch biopsies taken from the ear. UV DNA anti-sera were obtained from two sources; (1) From a separate group of rabbits previously immunized with UV DNA whose serum contained high titre antibody to UV DNA which was shown by inhibition studies not to cross react with native DNA. (2) From patients with SLE who had previously been found to possess a high titre of antibody to UV DNA in addition to a high titre of antibody to native DNA.

In vitro studies of the binding of both native and UV DNA to basement membrane at the dermal-epidermal junction were performed using skin biopsies taken from normal rabbits and three normal human controls.

METHODS

This study was divided into two parts. Study A—Cryostat sections of skin biopsies from normal rabbits and those exposed to ultraviolet light were incubated with (1) human antisera to UV DNA and subsequently with fluoresceinated goat anti-human immunoglobulin. (2) Human UV DNA antisera alone. (3) Fluoresceinated goat anti-human immunoglobulin alone. After washing, sections were examined by an incident light fluorescence microscope (Leitz Orthoplan). Evidence of positive fluorescent staining both in the dermal nuclei and at the dermal-epidermal junction was sought.

Study B—Skin biopsies from normal rabbits and normal human controls were prepared as before. Sections were incubated with preparations of either native DNA or UV DNA. Each section was washed three times in PBS and treated either with antisera to native DNA obtained from patients with SLE and a goat anti-human conjugate or with rabbit antiserum to UV DNA plus a goat anti-rabbit conjugate. Each section was examined for fluorescent staining at both the dermal-epidermal junction and the dermal nuclei.

In both studies the histological appearances were interpreted without knowledge of the source of tissue or antiserum.

RESULTS

Study A—The effect of ultraviolet light on both dermal nuclear DNA and its release at the dermal-epidermal junction are summarized in Table 1. In those rabbits exposed to ultraviolet light, UV DNA

TABLE 1. Effect of UVL *in vivo* on the release of UV DNA and deposition of immunoglobulin at the dermal-epidermal junction

	Human UV DNA antisera and goat anti-human conj.		UV DNA antisera		Goat anti-rabbit conj.	
	Nucleus	DEJ	Nucleus	DEJ	Nucleus	DEJ
UVL exposure	+	+	Neg.	Neg.	Neg.	+
Controls	±	Neg.	Neg.	Neg.	Neg.	Neg.

not only could be detected in the dermal nuclei but also was seen deposited in a linear fashion at the dermal-epidermal junction. No such changes were seen in those sections incubated with UV DNA antisera alone. When sections of UV irradiated skin stained with goat anti-rabbit conjugate alone were examined, there was no evidence of nuclear staining but some evidence of staining of the dermal-epidermal junction. In those rabbits not exposed to ultraviolet light some fluorescent staining of the nuclei was seen using the human UV DNA antisera and goat anti-human conjugate but none seen at the dermal-epidermal junction. No staining of nuclei or dermal-epidermal junction was seen either with UV DNA antisera alone or with goat anti-rabbit conjugate alone.

Study B—These results are summarized in Table 2. When sections of normal skin were incubated with native DNA followed by a human serum containing antibodies to native DNA and a goat anti-human conjugate, native DNA was detected not only in the nucleus but also bound to the dermal-epidermal junction. No such changes were seen when sections were treated with a rabbit serum containing antibodies to UV DNA and goat anti-rabbit conjugate. When sections were incubated with UV DNA positive staining at the dermal-epidermal junction and the nucleus was also seen with human antisera to native DNA and conjugate thus reflecting the cross reactivity of the antibody to native DNA with UV DNA. When the sections were treated with rabbit antisera to UV DNA and conjugate, staining was seen only at the dermal-epidermal junction. No nuclear staining was detected.

In control sections positive fluorescence was detected only in the nuclei using the human antiserum containing antibodies to native DNA.

TABLE 2. Binding of n-DNA and UV DNA to the dermal-epidermal junction *in vitro*

Tissue substrate incubated with	Human antisera to n-DNA + conj.		Rabbit antisera to UV DNA + conj.	
	DEJ	Nucleus	DEJ	Nucleus
n-DNA	Pos.	Pos.	Neg.	Neg.
UV DNA	Pos.	Pos.	Pos.	Neg.
Control	Neg.	Pos.	Neg.	Neg.

DISCUSSION

The role of UV light in the pathogenesis of systemic lupus erythematosus remains unclear. As photodimerization of native DNA to UV DNA produces a substance which is highly immunogenic it has been suggested that this may be one of the mechanisms by which tolerance to native DNA is broken in patients with SLE (Levine *et al.*, 1966). To support this suggestion, antibodies to UV DNA have been detected in patients with SLE and have shown to cross react with native DNA (Davis, Russell & Percy, 1976). These antibodies, however, correlate poorly with the presence or absence of a history of photosensitivity. Nor in experimental animals do antibodies induced to UV DNA cross react with other polynucleotides, suggesting that more than one factor is involved in SLE.

The demonstration that *in vivo* conversion of native DNA to UV DNA can occur in the dermis (Tan & Stoughton, 1969) again supports the possible role of ultraviolet in the pathogenesis of SLE as does the demonstration of experimentally induced skin lesions and nephritis in experimental animals presensitized to UV DNA (Natali & Tan, 1972; Natali & Tan, 1973). Our study has confirmed the observations of Natali & Tan that in experimental animals immunized to UV DNA and exposed to ultraviolet light, immunoglobulin deposits can be detected in skin biopsies, suggesting the release of an antigen at the dermal-epidermal junction. Our studies have also confirmed that conversion of native DNA to UV DNA does occur after such exposure. We have also been able to demonstrate using a human antiserum containing antibodies to both native and UV DNA that UV DNA may be released at the dermal-epidermal junction and may serve as the antigen for such immunoglobulin deposition. That this deposition is dependent upon the presence of ultraviolet light is suggested by the fact that no such change could be detected in experimental animals immunized to UV DNA but not exposed to ultraviolet light. Under normal circumstances, exposure to ultraviolet light and the production of UV DNA would result in the activation of the normal UV DNA repair mechanism and the rapid removal of this potential antigen. Why this does not occur both in patients with SLE and in experimental animals is not fully explained. In SLE, however, there has been a suggestion that the UV DNA repair mechanism may be defective leading to the persistence of the antigen (Hadjayannaki & Lachmann, 1972; Beighlie & Teplitz, 1975). Another factor may be persistence of the antigen at its site of release due to binding to the basement membrane at the dermal-epidermal junction. Such binding may be initiated by the basement membrane or collagen which has previously been shown to have a high affinity for DNA in studies performed on renal glomerular tissue (Izui, Lambert & Miescher, 1976). Our *in vitro* studies would further support this hypothesis. We were able to demonstrate using normal skin from both rabbits and humans that UV DNA and native DNA will bind to the dermal-epidermal junction with the production of histological changes similar to those seen in patients with SLE. The avidity of such binding may be responsible for the failure of the dissemination of antigen in SLE and its localization at the dermal-epidermal junction.

Our studies do not suggest that ultraviolet light induction of UV DNA is the sole or even the most important factor in the pathogenesis of SLE. These results lend support to the hypothesis that ultraviolet light induction of UV DNA and its subsequent release at the dermal-epidermal junction may be an important part in the induction or exacerbation of SLE following exposure to sunlight.

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REFERENCES

- BEIGHLIE, D.J. & TEPLITZ, R.L. (1975) Repair of UV damaged DNA in systemic lupus erythematosus. *Journal of Dermatology*, **2**, 149.

- DAVIS, P., RUSSELL, A.S. & PERCY, J.S. (1976) Antibodies to UV light denatured DNA in systemic lupus erythematosus. *Journal of Rheumatology*, **3**, 375.
- DAVIS, P. (1977) Antibodies to UV DNA and photosensitivity. *British Journal of Dermatology*, **97**, 197.
- DUBOIS, E.L. (1974) *Lupus Erythematosus*, 2nd edn. University of Southern California Press, Los Angeles
- HADJAYANNAKI, K. & LACHMANN, P.J. (1972) The relation of deoxyribonuclease inhibitor levels to the occurrence of antinuclear antibodies in NZB/NZW mice. *Clinical and Experimental Immunology*, **11**, 291.
- IZUI, S., LAMBERT, P.M. & MIESCHER, P.A. (1976) *In vitro* demonstration of a particular affinity of glomerular basement membrane and collagen for DNA. A possible basis for a local formation of DNA-anti DNA complexes in SLE. *Journal of Experimental Medicine*, **144**, 428.
- LEVINE, L., SEAMAN, E., HAMMERSCHLAG, E. & VAN VUNAKIS, H. (1966) Antibodies to photoproducts of DNA irradiated with ultraviolet light. *Science*, **153**, 1666.
- NATALI, P.G. & TAN, E.M. (1972) Experimental renal disease induced by DNA-anti DNA immune complexes. *Journal of Clinical Investigation*, **51**, 345.
- NATALI, P.G. & TAN, E.M. (1973) Experimental skin lesions in mice resembling systemic lupus erythematosus. *Arthritis and Rheumatism*, **16**, 579.
- TAN, E.M. (1968) Antibodies to deoxyribonucleic acid irradiated with ultraviolet light: Detection by precipitins and immunofluorescence. *Science*, **161**, 1353.
- TAN, E.M. & STOUGHTON, R.B. (1969) Ultraviolet light alteration of cellular DNA *in vivo*. *Proceedings of the National Academy of Science (USA)*, **62**, 708.

Case Reports

Oesophageal involvement in bullous pemphigoid

A POSSIBLE CAUSE OF GASTROINTESTINAL HAEMORRHAGE

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SUMMARY

An elderly patient with bullous pemphigoid who had extensive oesophageal bullae is described. The diagnosis was confirmed by routine histopathological examination and immunofluorescence studies. These large oesophageal bullae may have caused massive gastrointestinal haemorrhage in this patient.

Bullous pemphigoid is a blistering skin disease of the elderly. Oesophageal mucosal involvement, although infrequently reported, does occur and may be extensive enough to slough off the entire epithelial surface (Foroozan *et al.*, 1967). Despite this characteristic, haemorrhage or extensive blood loss has not been an associated complication of bullous pemphigoid of the oesophagus. The following case report describes, for the first time to the best of our knowledge, bullous pemphigoid of the oesophagus as the probable cause of an acute upper gastrointestinal haemorrhage.

CASE REPORT

An 84-year-old white female entered Milwaukee County General Hospital on April 1, 1977 with a 2 week history of a pruritic, blistering skin eruption. Initially, the blisters affected her arms. Gradually involvement spread to the trunk and lower extremities. The patient complained of throat pain and dysphagia for solids from the onset. There was no previous history of skin eruption, and her general health was unremarkable except for mild hypertension and diet-controlled maturity onset diabetes.

On physical examination, the patient was obese, in moderate distress, and had extensive blistering lesions over the integument. There were numerous tense and flaccid bullae predominately on the extensor surface of the upper extremities, the neck, inframammary folds, groins, buttocks, and thighs. Large tense bullae were also noted on the palms. Some bullae were haemorrhagic. Oral or ocular lesions were not noted during the entire course of her illness. A gynaecological examination did not reveal any vaginal bullae. Skin biopsies showed subepidermal microvesicles. In some areas large subepidermal bullae (or separation) were noted. Direct immunofluorescence of the skin lesions,

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performed by standard techniques (Beutner *et al.*, 1970), revealed linear deposits of C3 at the dermo-epidermal junction (Fig. 1) while indirect immunofluorescence study of the patient's serum was negative.



FIGURE 1. Linear deposition of C3 at the dermo-epidermal junction. This is direct immunofluorescence.

Initial treatment consisted of hydroxyzine, potassium permanganate compresses and topical steroids pending results of biopsies and immunofluorescence studies. On 4 April 1977, the patient developed massive haematochezia, syncope, and a drop in the haematocrit from 42% to 37.5%. Shortly thereafter she was endoscoped.

At the time of endoscopy, gastric lavage disclosed copious amounts of fresh and clotted blood. Examination was first attempted with an adult fiberoptic endoscope. Because of the extensive oral pharyngeal ulceration and oedema which compromised the proximal oesophageal lumen, a paediatric endoscope (9 mm diameter) was necessary. The oesophagus was intubated under direct vision. Fresh blood was noted in the distal segment of the oesophagus. Throughout the oesophagus, but especially in the proximal half, large egg-shaped vesicular lesions were noted to protrude from the oesophageal mucosa into the lumen. These lesions ranged in size from 0.5 to 1.0 cm in diameter. They were violaceous in colour, appeared tense with preservation of the overlying mucosa. In the distal half of the oesophagus, where fresh blood was pooled, several bullous lesions of various sizes were noted (Fig. 2). There was no evidence of oesophagitis, varicosities or other mucosal abnormality. The stomach was remarkably clear of blood, and the mucosa here, as well as in the duodenal bulb and the descending portion of the duodenum, was entirely normal. It was thought that the patient was bleeding from the distal oesophagus and from one or several ruptured lesions.

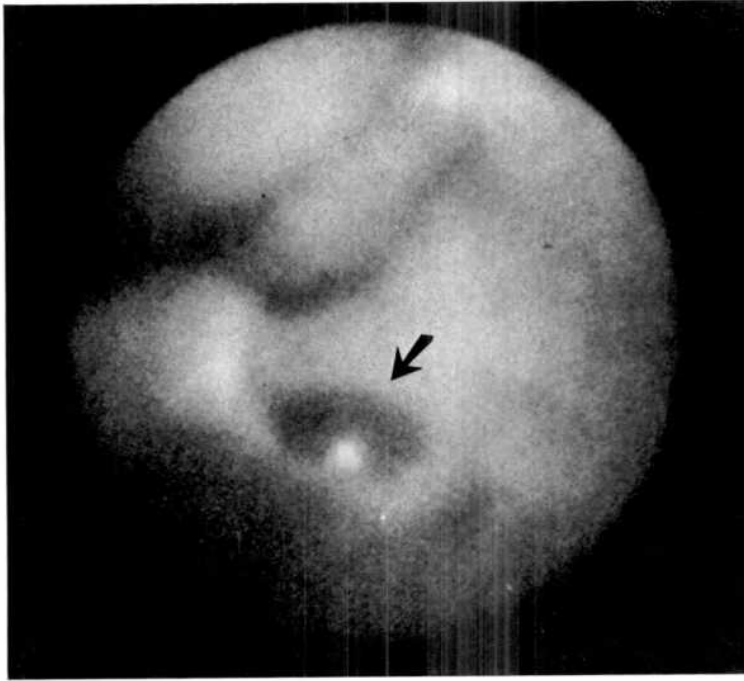


FIGURE 2. A haemorrhagic bulla located on the distal oesophageal mucosa was visualized under endoscopy.

The patient was transfused with packed red cells, and the haematocrit stabilized within 24 h. Systemic corticosteroids were started. There was some improvement in the bullous pemphigoid of the skin, and at the same time the difficulty and pain on swallowing began to subside. An oesophagram done 8 days later showed only slight irregularity of the luminal contour of the proximal oesophagus suggesting persistence of these lesions.

On the 13th day, the patient appeared obtunded and her temperature rose to 101°F. Despite antibiotics and supportive care, she became increasingly lethargic and septic, lapsed into a coma, and died 15 days after admission. An autopsy could not be obtained.

DISCUSSION

Oesophageal involvement in bullous pemphigoid is probably more common than previously reported. The oesophageal epithelium, like the remaining gastrointestinal tract, has a very rapid rate of regeneration. Soon after bulla formation, new epithelium regenerates above the basement membrane, accounting for the frequent intraepidermal location of the blister on biopsy. This fact may explain why most patients with this disease are asymptomatic as far as oesophageal manifestations.

Cutaneous haemorrhagic bullae are frequently seen in this disease especially after minor trauma, e.g. scratching. Mechanical or chemical irritation from ingestants could account for haemorrhage into oesophageal bullae. Conceivably a small amount of haemorrhage would not have any clinical significance. The reason why our patient had such copious haemorrhage is speculative. Either her advanced age or debilitated state may have precluded adequate and rapid epithelial regeneration or

certain bullae were located in close proximity to superficial blood vessels. Even though we cannot be entirely certain that our patient bled from a ruptured oesophageal bulla, there was no other upper gastrointestinal lesion to suggest an alternative source for the bleeding.

We assume that the oesophageal bullae were pemphigoid lesions because of the presence of concurrent cutaneous lesions. Clinically the onset of oesophageal symptoms coincided with the cutaneous eruption, and as the cutaneous bullae were improving with treatment, so did the difficulty and pain on swallowing. Most important, the oesophageal lesions appeared macroscopically similar to the skin lesions and were exclusively limited to the stratified squamous epithelium of the gullet and oral pharynx.

The oesophagus may be involved with bullous pemphigoid more frequently than is now appreciated. This should be suspected in patients with this cutaneous disorder who complain of dysphagia, or manifest gastrointestinal bleeding.

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We wish to thank Dr Marvin D. Miller of West Allis, Wisconsin, for interpreting the histopathological slides.

REFERENCES

- BEUTNER, E.H., CHORZELSKI, T.P. & JORDON, R.E. (1970) *Autosensitization in Pemphigus and Bullous Pemphigoid* (Monograph), p. 194. Charles C. Thomas Co., Springfield, Illinois.
- FOROZZAN, P., ENTA, T., WINSHIP, D.H. & TRIER, J.S. (1967) Loss and regurgitation of the esophageal mucosa in pemphigoid. *Gastroenterology*, **53**, 548.

Malignant pyoderma

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SUMMARY

A patient is described who developed multiple areas of inflammatory pyoderma on the face, leading to extensive ulceration. Repeated investigations failed to demonstrate any specific bacteria or fungus responsible and trials of treatment with various antibiotics proved unsuccessful. Histology revealed a granulomatous abscess-like lesion, and the condition resembled what has been described as malignant pyoderma. The patient was successfully treated with oral dapsone and intralesional steroids.

CASE REPORT

In July 1975 while on holiday in Spain a 47-year-old woman developed a dark red, papular lesion above her right zygoma. This gradually enlarged and developed a central crusted ulcer. When seen in September of that year the lesion was thought to be a kerato-acanthoma and was curetted. However, the lesion failed to heal and a further similar ulcerated lesion developed on the left side of her forehead (Fig. 2) and later multiple papular lesions over the left zygoma.

The primary lesion was now ulcerated and extremely tender (Fig. 1). The border was elevated and indurated and the surrounding tissue deeply undermined. A tail of undermined epithelium extended for 5 cm inferiorly over the zygoma from which a purulent material exuded. The ulcer base was necrotic and purulent. Apart from these lesions the patient had no other complaints and on examination was physically well.

Because of the purulent nature of the lesions, attempts were made to culture specific organisms. However, culture for acid-fast bacilli and leishmaniae as well as routine cultures proved sterile on several occasions. A biopsy of the lesion (Fig. 3) showed the appearances of a granulomatous reaction in the dermis with pseudoepitheliomatous hypertrophy. Inflammatory cells present included neutrophils, eosinophils, lymphocytes, histiocytes, a few plasma cells, epithelioid cells and giant cells—mostly of Langhans type. There were small areas of necrosis present. No bacilli, fungi or parasites were seen with ZN, PAS and Giemsa stains. Full blood count, biochemistry and chest X-ray were normal. Serological tests for syphilis and rheumatoid factor were negative and antinuclear antibodies were insignificant. Direct and indirect immunofluorescence was negative and serum immunoglobulins were normal apart from a slightly lowered IgA. Sigmoidoscopy and rectal biopsy were normal.

In spite of a lack of laboratory evidence of infection, because the lesions were apparently purulent, trials of antibiotics were given. Three 10 day courses of metronidazole initially appeared to promote healing, and 4 months after cessation of therapy atrophic scars had formed over the original ulcers.

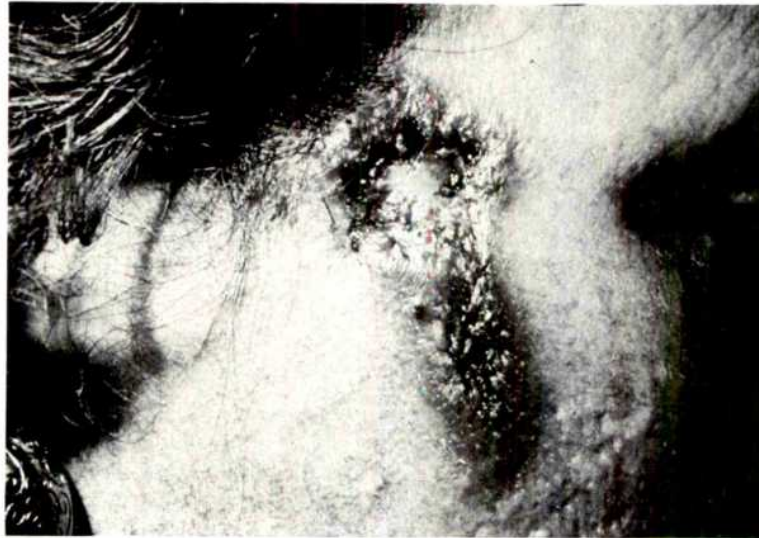


FIGURE 1. Lesion on right temple.

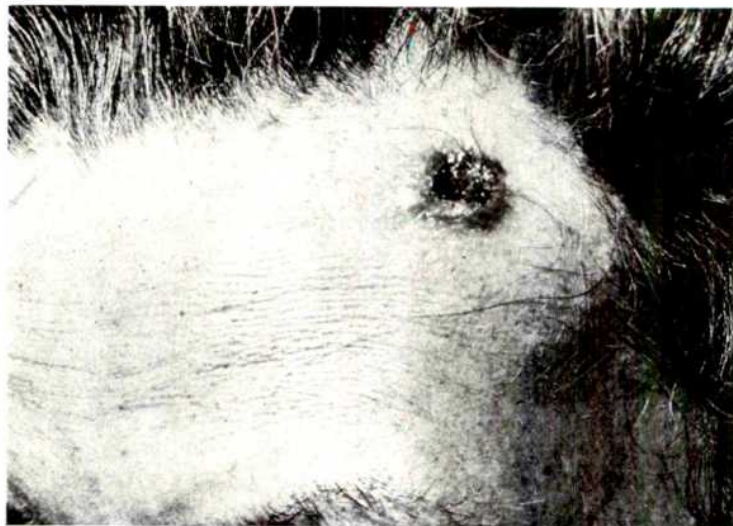


FIGURE 2. Lesion on left side of forehead.

However, only 3 weeks elapsed before ulceration recurred. During the time that these ulcers were healing, multiple hard painful papules appeared on the left cheek, one of which became ulcerated. Over the next 3 months a repeated trial of metronidazole and later trials with tetracycline, clindamycin, rifampicin and isoniazid failed to alter the clinical appearance of the lesions. Repeated investigations failed to provide new evidence of aetiology.

It was unlikely, therefore, that infection played any part in this patient's condition and it was thought that the disease process could be an abnormal manifestation of pyoderma gangrenosum. At this time the diagnosis of malignant pyoderma was suggested.

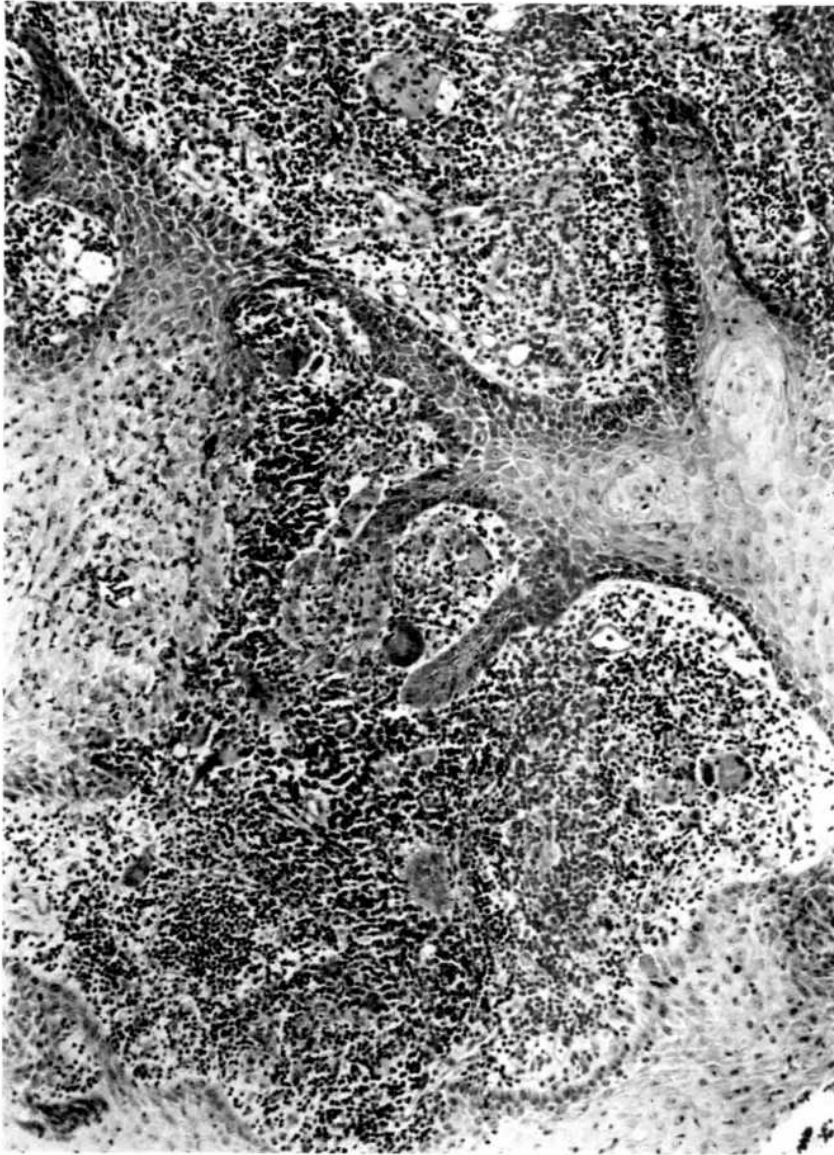


FIGURE 3. Section from border of ulcer showing mixed inflammatory cell infiltrate and many giant cells (H & E, $\times 150$).

In January 1977 the patient was commenced on therapy with dapsone 200 mg daily and the individual lesions were injected weekly with triamcinolone by Dermojet. From that time no new lesions appeared and the old lesions began to heal. Therapy was gradually withdrawn and was terminated in June 1977 when all the lesions had healed, the previously ulcerated areas being covered with atrophic scar tissue. In November 1977 there had been no further recurrence.

DISCUSSION

The purulent nature of this patient's lesions suggested an infective cause; however, repeated bacteriological investigations failed to support this hypothesis. Eleven months prior to developing the first pustule, the patient had been on holiday in Italy, an area where we thought she might possibly have contacted cutaneous leishmaniasis. The subsequent course of the disease and the negative bacteriological findings made this highly unlikely.

Pyoderma gangrenosum was considered, but it was thought that with the acute onset and rapid advancement of the lesions it did not conform to the recognized clinical pattern of this disease. Nor was there any evidence of underlying systemic inflammation such as ulcerative colitis.

The suggestion that this patient's condition closely resembled three cases described by Perry *et al.* in 1968 as malignant pyoderma was welcomed. Perry and his colleagues described an inflammatory pyoderma involving predominantly the face, neck and upper trunk in which there was marked tissue destruction.

In the cases described, no specific infective cause could be found and there was no response to systemic antibiotic therapy. Systemic corticosteroids in large dosage were necessary to arrest the course of the disease. There was concern, however, that continued large doses of systemic steroids given to a woman in this age group could give rise to further problems. Local injection of the lesions with triamcinolone was therefore considered as this had been shown to be successful in the treatment of pyoderma gangrenosum by Moschella in 1967.

The concurrent administration of dapsone was thought to be a necessary precaution, as obviously only pre-existing lesions could be treated by local injection with steroids and dapsone has been shown to be of value by Soto (1970) as well as other workers in treating pyoderma gangrenosum.

Although this condition has been termed malignant pyoderma, there is no evidence of neoplastic change. The description 'aggressive idiopathic granulomatous pyoderma' may therefore be more appropriate.

ACKNOWLEDGMENT

I wish to thank Dr C.D.Calnan of St John's Hospital, London, for suggesting the diagnosis of malignant pyoderma.

REFERENCES

- MOSCHELLA, S.L. (1967) Pyoderma gangrenosum: A patient successfully treated with intralesional injection of steroid. *Archives of Dermatology*, **95**, 121.
PERRY, H.O., WINKLEMAN, R.K., MULLER S.A. & KIERLAND, R.R. (1968) Malignant pyoderma. *Archives of Dermatology*, **98**, 561.
SOTO, L.D. (1970) Diaminodiphenylsulfone and steroids in the treatment of pyoderma gangrenosum. *International Journal of Dermatology*, **9**, 293.

James Startin, Jonathan Hutchinson and the Blackfriars Skin Hospital

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The very rapid increase in the population of London in the latter half of the eighteenth, and throughout the nineteenth century overwhelmed the limited facilities for medical care provided by the old hospitals of St Bartholomew and St Thomas, and the eighteenth-century foundations of similar character, the Westminster, St George's, Guy's and the London Hospitals. The fees of apothecaries and surgeons in general practice were beyond the reach of most of the sick poor and the vast numbers were beyond the capacity of the Poor Law system of medical relief administered by the officers of the parish. In response to this urgent need general dispensaries were established in London and in other large cities from 1770 onwards (1). Such dispensaries were in effect general medical and surgical outpatient clinics, subsidised by private charity. Many provided home visits for patients too ill to come to the dispensary. Some of them attracted medical staff of high calibre.

Dermatology as a speciality did not exist, but surgeons in their routine courses often devoted several lectures to diseases of the skin. Physicians such as Robert Willan, and Thomas Bateman, became interested in skin disease because of its frequency in dispensary practice, and because of the importance of the exanthematic fevers in the overcrowded courts and alleys of London. Some dispensaries, including that in Carey Street (founded 1782), where Willan and Bateman worked, acquired a local and eventually a national reputation for dermatology, but they remained general dispensaries. Anthony Todd Thomson one of Bateman's pupils, who was later to establish at the institution which became University College Hospital the first dermatology clinic at a London teaching hospital, became interested in dermatology (2) because he saw so many patients with skin disease at the Chelsea Brompton and Belgravia dispensary in Sloane Square, which he was partly instrumental in founding in 1812. Later this dispensary too acquired some reputation in dermatology.

New general dispensaries, charitable or provident (3), continued to be founded throughout the century, and were often established and staffed by graduates of Scottish universities—Willan, Bateman and Thomson are typical examples—who found themselves excluded from the conservative and inbred teaching hospitals. From the late eighteenth century, but in ever greater numbers in the nineteenth, hospitals and dispensaries were opened for the treatment of special classes of diseases or special sections of the population; hospitals for example for the eye or skin diseases, and hospitals for children (4). The founders were usually doctors and, as was the case in the general dispensaries, often Scottish graduates, although later in the nineteenth century many other medical men, some of them excluded for one reason or another from the traditional channels of professional advancement, chose this means of acquiring experience, and an entry into practice. The opponents of special hospitals,

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and they were numerous, vociferous and merciless, claimed that they were unnecessary and that their founders were motivated solely by self interest. The second charge cannot be totally refuted; some few of the hospitals were certainly designed to advance their founder's reputation, but some which originated in this fashion later developed into respectable institutions and lived down their past. A very few remained the private preserve of one man and usually failed to survive his retirement or death. The charge that the special hospitals were unnecessary was manifestly absurd; they attracted large numbers of patients for whom no satisfactory provision was made in the teaching hospitals or general dispensaries and they offered unique opportunities for the training of a new generation of specialists. The skin hospitals of London and those general dispensaries reputed in dermatology trained the men who were later to bring dermatology into the teaching hospitals.

The skin hospitals of London are listed in Table 1. The earliest, 'The London and Westminster Infirmary for The Treatment of Cutaneous Diseases', was founded in Great Marlborough Street in 1819 (5) and was usually referred to as the Marlborough Street Dispensary for Diseases of the Skin. Bateman was consulting physician, and Thomson was one of the physicians.

All the hospitals and dispensaries listed in the table have played a part, in some instances admittedly a modest one, in the development of dermatology in Britain. Two of them however have been of outstanding importance; Blackfriars and St John's. James Startin at Blackfriars trained Jonathan Hutchinson in dermatology, and he in turn trained there a succession of dermatologists over many decades. From 1841, for some seventy or eighty years, it was the leading postgraduate centre for dermatology in Britain and its staff included some of the greatest British dermatologists of the period. Only after 1920 did the status of St John's Hospital begin to rise, and that of Blackfriars relatively to decline.

TABLE 1. The skin hospitals of London

1819	Royal London and Westminster Infirmary for the Treatment of Cutaneous Diseases, Great Marlborough Street
1841	Hospital for Diseases of the Skin, London Wall (later moved to Blackfriars)
1841	Dispensary for the Treatment of Ringworm and other Diseases of the Skin, Princes Street, Leicester Square
1843	West London Free Dispensary for Diseases of the Skin, Dorset Street
1844	Dispensary for Diseases of the Skin, Blenheim Street
c.1850	Dispensary for Diseases of the Skin, Hampstead Street
1851	Western Dispensary for Diseases of the Skin, Great Portland Street (from 1889 Western Hospital)
1857	London Dispensary for Diseases and Ulceration of the Legs, Bloomsbury Square
1863	St John's Hospital for Diseases of the Skin
1864	British Hospital for Skin Diseases, Great Marlborough Street
1864	National Free Institution for Diseases of the Skin, Grays' Inn Road (later known as the Central London Institution)
1866	City Hospital for Diseases of the Skin, City Road
1887	Skin Hospital, Fitzroy Square
1898	St Paul's Hospital for Skin and Genitourinary Diseases, Red Lion Square

JAMES STARTIN

James Startin was born in 1806 at Mosley, near Birmingham, where his father was a merchant and banker. On leaving school he was apprenticed to two general practitioners at Atherstone, after which he worked as a surgical dresser to Hodgson (6) at the Birmingham Hospital, before spending two years as a student at St Bartholomew's Hospital. He was still too young to qualify so he worked for a

year as assistant in a colliery practice at Walsall, before returning to St Bartholomew's as a pupil of J.P. Vincent the surgeon. He attended Henry Clutterbuck's lectures on medicine at the General Dispensary, Aldersgate Street, and also Sir Charles Bell on surgery and Quain on anatomy. He qualified L.S.A. 1827, M.R.C.S. 1828, and after a few months in general practice was appointed resident surgeon to the Birmingham Town Infirmary, and held this post for three years. He then spent two years as the house surgeon to the Birmingham General Hospital. He set up in general practice in Warwick, but although he was successful he was soon forced to sell his practice to meet the debts which followed the failure of a local bank in which he had many shares. He left England and spent between two and three years in France, mainly at the Hôpital St Louis in Paris, but also at Montpellier. At the Hôpital St Louis he is likely to have attended the lectures and clinics of Bielt (7). A contemporary English visitor wrote; 'his lectures were good and very practical; following Willan and Bateman's arrangement, and always speaking in high terms of the general eligibility and success of English modes of treatment' (8).

On his return to England he started practice as a surgeon in London, with a special interest in dermatology, to which his work was before long confined.

The *Lancet* obituarist wrote (9): 'The fame of the deceased as a dermatologist was widely spread, and his clientele was certainly not surpassed in numbers by any other surgeon of the day'. In his private consulting room he saw from forty to fifty patients in a day. He was a sociable, genial, kindly man who owed his immense reputation to his mastery of the limited therapeutic agents and procedures then available. 'He believed in curing people of their maladies and in this object he was successful to a very unusual extent.' Startin's outstanding success in the management of skin disease is confirmed by the surgeon T.B. Curling, whose duty it was as President of the Royal Medicochirurgical Society to include in his annual address an obituary tribute to Startin (10). C.J.B. Williams (1845-1859), Professor of Medicine at University College, wrote of Startin: 'a dear and valued friend, . . . his treatment in his special department, diseases of the skin, was far more successful than that of any practitioner that I ever knew. His knowledge may not have been very scientific, or based on very exact pathological investigation, but he succeeded in curing exacerbated and inveterate forms of skin disease that had baffled many other practitioners. He was the first to introduce the free use of glycerine in medicine; and having traced acne and other follicular diseases to fatty concretions, he successfully applied oil of turpentine and other essential oils to disperse them. He was remarkably ingenious and fertile of resources, in the use of caustics and in small surgical operations . . . I could not always equally approve of his internal remedies' (8).

Williams mentions Startin's empirical approach, and in this criticism Curling and the *Lancet* concur. His main publication was a course of lectures which appeared in the *Medical Times* in 1846. They are conventional and unoriginal. Nevertheless, 'when he was in his prime, a diligent class was usually to be found in the Blackfriars outpatient room.' It is as a clinical teacher that Startin deserves to be remembered. His most famous pupil was Jonathan Hutchinson, but Thomas Hillier, predecessor of Tilbury Fox at University College, was also trained at Blackfriars (11). Hutchinson later recalled (12) that for a great many years this hospital was the only one in Britain at which teaching and experience in dermatology could be obtained.

Startin died on December 24th 1827 at his country home at Woodford in Essex, a few days after an operation for a stone in the bladder.

THE HOSPITAL FOR DISEASES OF THE SKIN, BLACKFRIARS (13)

On his return from France, Startin conceived the ambition of establishing in London a skin hospital, which might in time come to rival the Hôpital St Louis in Paris. He eventually secured the patronage

of the Royal Dukes of Cambridge and of Sussex, and the financial support of Samuel Gurney, the Quaker banker and philanthropist. A modest hospital, at first known as the London Cutaneous Infirmary, opened at London Wall in 1841, but moved to larger premises at Blackfriars after a year or two, and changed its name to the Hospital for Diseases of the Skin. Startin appears to have run the hospital single-handed until 1851, although Thomas Hodgkin and Southwood Smith were soon listed as honorary physicians, and continued in that capacity until they died. Hodgkin is remembered as a pathologist and Southwood Smith as a sanitary reformer, but both earned their livings as practising physicians.

In 1851 A.M. McWhinnie (1807–1866) joined the staff of the hospital as assistant surgeon; he lived very near the hospital. He was an anatomist at St Bartholomew's Hospital, where he was elected assistant surgeon in 1854. His health began to fail in 1860 but he remained on the staff at Blackfriars and was appointed full surgeon there in 1866, in which year he died. By 1859 the average number of patients attending the hospital was 10,000. Startin could have dealt effectively with such numbers only with the help of many assistants. The names of these assistants are known only if they were subsequently elected to the staff. One such was George Burt (1789–1874) who qualified in 1810 from the United Hospitals (14). He worked in surgical practice in London and was F.R.C.S. in 1852. He attended regularly at the Skin Hospital and was elected assistant surgeon in 1860 and surgeon in 1867.

When Jonathan Hutchinson first began to attend the hospital, and when he began to undertake a share of the work, is unknown. Hutchinson, a Quaker, was born at Selby, Yorkshire, in 1828. He served a surgical apprenticeship in York and went to London in 1850 to complete his training at St Bartholomew's Hospital, where his teachers included Startin's colleague McWhinnie. He qualified M.R.C.S., L.S.A. later in 1850, and then returned to York as house surgeon to the County Hospital. In 1851 he was back in London studying ophthalmology at Moorfields, and learning all he could in all branches of medicine and surgery. Little is known about his activities during the next five years (15); he certainly had some private means, and his requirements were modest. It is known that he made contact with fellow Quakers in medical practice such as Thomas Hodgkin of Guy's Hospital, who no doubt advised and encouraged him. He supplemented his income by medical journalism and did not begin to build up a practice until 1856. Considering the immense knowledge of diseases of the skin which he later displayed it seems justifiable to assume that during these years he spent much time at Blackfriars. Either McWhinnie or Hodgkin could have introduced him to Startin. From 1867 he was on the active staff and later the consulting staff of the Skin Hospital until his death in 1913. His great reputation ensured that other dermatologists from teaching hospitals would join him there. Hutchinson had been elected to the staff of the London Hospital in 1859. The skin hospitals whose staff lacked such teaching hospital affiliations in general failed to achieve the prestige of Blackfriars.

NOTES AND REFERENCES

- (1) The first was the Aldersgate Street Dispensary.
- (2) A short biobibliography of A.T. Thomson appears in *Willan Library Bulletin III*, issued with *British Journal of Dermatology*, 1978.
- (3) A provident dispensary was partly supported by small subscriptions from potential patients.
- (4) Fraser, F.R. (1964) The rise of specialisation and special hospitals. In: *The Evolution of Hospitals in Britain* (Ed. by F.N.L. Poynter), p. 168. Pitman, London.
- (5) Highmore, A. (1822) *Views of the Charitable Institutions established in and around London*, pp. 383–386. London.
- (6) Joseph Hodgson, F.R.S. F.R.C.S. (1805–1869) was a leading Birmingham surgeon.
- (7) Laurent Theodore Bielt (1781–1840) was born in Switzerland. A pupil of Alibert at the Hôpital St Louis,

he was largely responsible for the teaching there from 1815. In 1816 he attended Bateman's clinic in London.

- (8) Williams, C.J.B. (1880) *Memoirs of Life and Work*, p. 269. Smith Elder, London.
- (9) Anon. (1873) Obituary. *Lancet*, 1, 34.
- (10) Curling, T.B. (1871-5) Obituary of James Startin. *Proceedings of the Medicochurgical Society of London*, 7, 137.
- (11) Unna, P.G. (1902-3) The history of eczema in the last century in England. *Transactions of the Dermatological Society of Great Britain and Ireland* for May 22nd 1902.
- (12) At a dinner given in 1907 to mark the merging of the Dermatological Society of London and the Dermatological Society of Great Britain and Ireland to form the Section of Dermatology of the Royal Society of Medicine, Jonathan Hutchinson said that fifty years earlier only the Blackfriars Hospital offered facilities for the student of dermatology.
- (13) The Blackfriars Hospital was closed in 1948. The records of the hospital have not been traced despite a thorough search in London archives and libraries. It is to be hoped that they have not been destroyed for they are of great historical interest to dermatologists. In their absence the outline of the hospitals' history has been compiled from medical and local directories, in particular the various editions of Sampson Low's *Charities of London*.
- (14) St Thomas' Hospital and Guy's Hospital, at that time side by side, near London Bridge.
- (15) Hutchinson, H. (1946) *Jonathan Hutchinson*, pp. 33-38. Heinemann, London.

Brief Communication

Combined therapy with oral retinoid and PUVA baths in severe psoriasis

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Promising results of oral treatment of psoriasis with a synthetic derivative of vitamin A (Ro 10-9359*) have recently been reported (Dahl, Möllenbach & Reymann, 1977; Orfanos & Runne, 1976; Ott, 1977; Schimpf, 1976). During a clinical trial of this retinoid, we observed a good response in three out of seven patients with severe psoriasis. On the other hand, three of the patients experienced a worsening of their psoriasis after a few weeks. These three patients and one with insufficient effect of retinoid alone were subsequently treated with trioxsalen baths and UV-A light (PUVA baths) with rapid healing. When PUVA baths alone had been tried earlier with three of the patients, this treatment was ineffective. The 'PUVA bath' method has previously been described by Fischer & Alsins (1976): a trioxsalen bath (0.5 mg/l) is followed by irradiation with dysprosium lamps.

In Table 1 the effects of the different treatments are outlined. The patients were all males and had a long history of psoriasis with insufficient response to all types of previous therapy, not having been free from psoriasis for several years. The patients were seen once every 2 weeks until PUVA treatment was started. Petrolatum was used once daily as topical treatment. During retinoid therapy, increased erythema, infiltration, and scaling of the old psoriatic lesions were observed and new lesions also appeared in three of the patients. The day after the unsuccessful retinoid therapy had been stopped, treatment with PUVA baths 5 days a week was initiated. The irradiation time was 15–25 s. It was not necessary to gradually increase this time as is usually the case during ordinary PUVA treatment (Fischer & Alsins, 1976). The patients were markedly improved after one or two treatments and they were completely healed or almost healed with 1–3 weeks of therapy. The healing time with PUVA baths alone is about 3–6 weeks (Fischer & Alsins, 1976). One patient (TF) had to stop treatment after 2 weeks due to non-medical reasons. In the other patients, the complete remissions lasted from 5 to at least 12 months without any treatment. In two patients (BS and GJ), the combined retinoid and PUVA treatment has been tried again with the same good results.

Like naturally-occurring vitamin A, retinoids are believed to participate in the regulation of mitotic

TABLE 1. Clinical details

Patient and age	Type of psoriasis	Retinoid dosage	Response to retinoid alone	Response to PUVA baths after retinoid therapy
BS (57)	Plaque type covering 60% of body	25 mg × 2 4 weeks	Worsening	Complete healing within 3 weeks. Remission for 8 months
JK (37)	Plaque type covering 35% of body	25 mg × 2 10 weeks	Worsening	Healed within 1 week. Remission for at least 12 months
GJ (29)	Nummular lesions on 50% of body	25 mg × 2 10 weeks	Worsening	Healed after 2 weeks. Remission for 5 months
TF (32)	Erythroderma	25 mg × 2 12 weeks	Moderate improvement	Nearly healed within 2 weeks. Remission for 6 weeks

* The preparation Ro 10-9359: ethyl all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate, was placed at our disposal by Hoffmann-La Roche Co. Ltd., Basle.

activity and in cellular differentiation of epithelial tissues (Bollag, 1974; Sporn *et al.*, 1976). A beneficial effect of oral treatment of psoriasis with retinoid alone was noted in some of our patients. The worsening of the psoriasis in others was stopped by subsequent PUVA baths that initiated a rapid healing of the lesions resulting in long remissions. We do not know the mode of action of the combined treatment with retinoid and PUVA baths. Preliminary results indicate that, on normal-appearing skin during the combined treatment, the minimum erythema dose of unfiltered light from the dysprosium lamp is lowered by 30–50% as compared with the dose required with trioxsalen alone (Michaëlsson *et al.*, unpublished observation). Studies are now underway to find out the optimal dosage of retinoid and PUVA in this new treatment of psoriasis.

REFERENCES

- BOLLAG, W. (1974) Therapeutic effect of an aromatic retinoid acid analog on chemically induced papillomas and carcinomas in mice. *European Journal of Cancer*, **10**, 731.
- DAHL, B., MÖLLENBACH, K. & REYMANN, F. (1977) Treatment of psoriasis vulgaris with a new retinoic acid derivate Ro 10-9359. *Dermatologica*, **154**, 261.
- FISCHER, T. & ALSINS, J. (1976) Treatment of psoriasis with trioxsalen baths and dysprosium lamp. *Acta dermatovenerologica*, **56**, 383.
- ORFANOS, C.E. & RUNNE, V. (1976) Systemic use of a new retinoid with and without local dithranol treatment in generalized psoriasis. *British Journal of Dermatology*, **95**, 101.
- OTT, F. (1977) Behandlung der Psoriasis mit einem oral wirksamen aromatischen Retinoid. *Schweizerische medizinische Wochenschrift*, **107**, 144.
- SCHIMPF, A. (1976) Zur systemischen Anwendung eines aromatischen Vitamin A-Säure-Derivates (Ro 10-9359) bei Psoriasis und Keratosen. *Schweizerische medizinische Wochenschrift*, **51**, 265.
- SPORN, M.B., DUNLOP, N.M., NEWTON, D.L. & SMITH, J.M. (1976) Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). *Federation Proceedings*, **35**, 1331.

Brief Communication

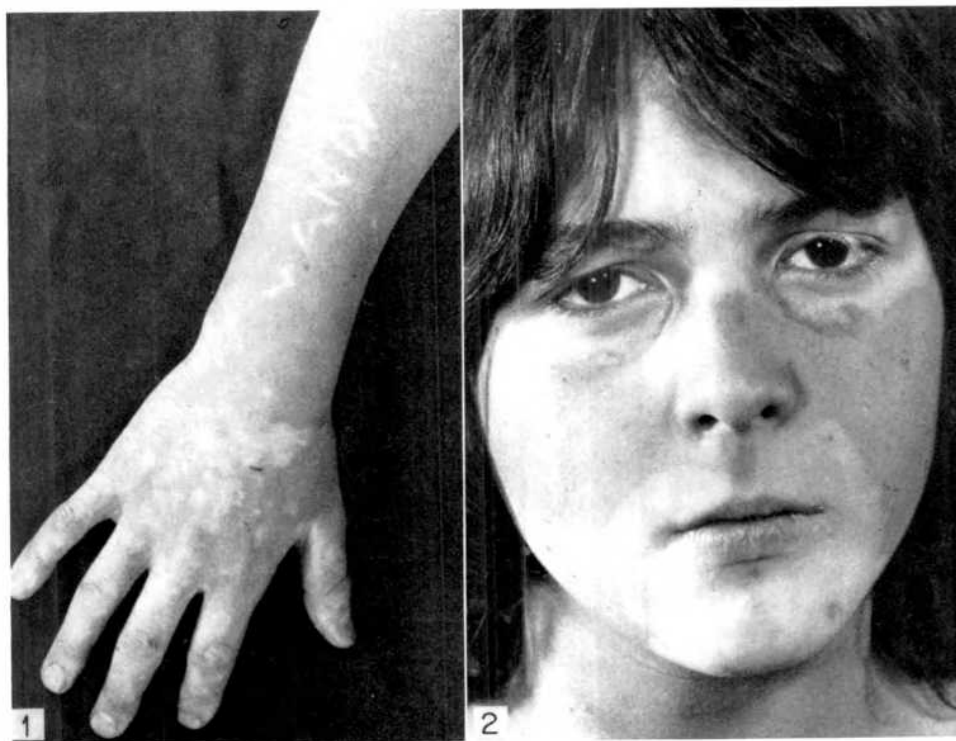
Vitiligo as a Köbner phenomenon

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Trauma has long been accepted as a localising factor in vitiligo, indeed it is mentioned in Hebra & Kaposi's textbook (1874) and later by Castellani & Chalmers (1913). Nevertheless, specific examples related to specific and timed injuries have seldom been recorded in what still remains an inexplicable quirk of human cutaneous behaviour.

It must, therefore, be of some interest that at about the time of his fourteenth birthday, young Nicholas took more than passing notice of a classmate called Mandy. He doesn't remember now where else he may have written her name and he doesn't really think about her very much any more, but he does remember idling away part of a geometry lesson scratching the magic letters with a pair of dividers on the extensor aspect of his right forearm—a little clumsily because he is right handed. He drew no blood but his devotion was evident for a day or two as a series of faint scratch marks with minimal linear crusts.

Nicholas is an extremely fit and active white boy with a capacity of cutaneous pigmentation common along the North Mediterranean littoral. He began to produce lesions of vitiligo in the spring of 1977, about 4 months after his temporary glimmerings of Mandy's charms. He found it a little unfair,



not to say embarrassing when, in addition to rather unattractive amorphous white areas on his chin, elbows and shins, and even in some of his eyelashes, the girl's name reappeared on his right forearm like some old film sent late to be developed.

In the succeeding 9 months more and more vitiliginous areas appeared on his legs and trunk but MANDY remains quite unchanged (Fig. 1).

The progression of the vitiligo in this case has been unusually rapid and is probably continuing. The involvement of the elbows, knees and shins suggests that trauma may have played some part in determining the sites of these patches, but lesions on the face and trunk and the occurrence of poliosis of the left upper eyelashes (Fig. 2) mark this as a typical case in a young person.

Numerous investigations were carried out and the only unusual finding was a positive anti-parietal cell antibody titre. Serum Vitamin B₁₂ and red cell folate levels were normal. There is no family history of vitiligo or of any type of anaemia.

ACKNOWLEDGMENT

The photographs are by Mr T. McCausland, Department of Medical Photography, Plymouth General Hospital.

REFERENCES

- CASTELLANI, A. & CHALMERS, A.J. (1913) *Manual of Tropical Medicine* 2nd edn, p. 1609. Baillière, Tindall, London.
- HEBRA, F. & KAPOSI, M. (1874) *Diseases of the Skin* (translated by W. Tay), Vol. 3, p. 180. New Sydenham Society.

Book Reviews

International Coding Index for Dermatology. Compatible with the 9th revision of the WHO International Classification of Disease. Compiled by SUZANNE ALEXANDER and ALAN B. SHRANK (1978). Published for the British Association of Dermatologists by Blackwell Scientific Publications, Oxford. Pp. 90. Price £2.00.

This booklet was commissioned by the British Association of Dermatologists and has just been published.

Dermatologie. Ein lexikalisches Repertorium. 3 Auflage. By KURT WINKLER (1978) Berlin, New York: De Gruyter. Pp. 318, 139 illustrations in black and white, 88 in colour. Price DM 98.

This is the third revised and updated edition of Professor Winkler's small reference book on skin and venereal disease to be published since 1968. It appears to be directed at senior medical students and general practitioners and would be particularly useful to dermatologists at the beginning of their training. As in previous editions the book is divided into three sections. The major part consists of short descriptions of the clinical features, differential diagnosis and treatment of some 1500 diseases. The second section lists these disorders according to the region of the body most frequently affected. This is followed by a comprehensive and useful subject index.

The entries in the first part of the book include some rarities (for example Haber's syndrome) as well as disorders of recent importance such as vinyl chloride disease. References to the literature are given, particularly where a disease is rare or has been recently described. The descriptions of the clinical features are admirably complemented by the many colour and black and white illustrations, most of which are excellent. Conditions to be considered in the differential diagnosis of each disorder are listed, but there is rarely any indication as to how one makes a distinction between them.

The sections on the management of skin disease tend to be rather didactic in places, and the lists of suggested remedies are sometimes too long to be of practical use. Although many important and up-to-date therapeutic measures are included, some of the treatments suggested would be considered ineffective, controversial or even potentially harmful by British dermatologists. There is a heavy reliance on supportive measures such as vitamins, tranquillisers, psychotherapy and climatotherapy. Sauna treatment is recommended for a variety of conditions ranging from keratosis pilaris to perniosis, and thallium is one of the many drugs suggested for the treatment of alopecia areata. It is difficult to understand the prohibition of all alcoholic drinks apart from red wine for patients with eczema and the recommendation of a low-salt diet in lichen planus.

Despite these occasional therapeutic vagaries, Professor Winkler has once again produced a reference book that is very comprehensive for its size, up-to-date, well-illustrated and easy to use. It can be recommended to students of dermatology and non-specialists alike and will undoubtedly enjoy the wide circulation it deserves.

NEIL SMITH

Leitfaden der Medizinischen Mykologie, 2nd Edn. By HERBERT KOCH (1977) Jena: V.E.B. Gustav Springer Verlag. Pp. 138, containing 75 illustrations including 12 colour plates. Price DDR 34.40M.

This is an excellent introduction to medical mycology which accomplishes exactly what the author claims to do, *viz.* present concisely the most important aspects of medical mycology to doctors, biologists and technicians. The first chapter deals with the place of the fungi in botanical classification followed by a section on the micro and macro morphology of fungi, presumably to familiarize the reader with the general terminology. I found the third sub-section on vegetative and sexual reproduction much clearer than many accounts I have read and it could possibly have been run in with the following section which introduces the concept of growth as parasites or saprophytes. Both of these sections are illustrated with excellent line drawings—some borrowed with due acknowledgment.

The second chapter of the manual deals with the mycoses—superficial, deep and systemic. I liked the way the author chose to deal with this section. He uses the clinical condition as the title followed by a list of infecting agents. A brief clinical description is given and then general information—incidence, sources of infection, etc. Finally, the most sensible way of approaching the diagnosis mycologically is given. The actual details of colony formation and microanatomy are left to later chapters (5 and 7).

Chapter 4 consists of two pages concerning erythrasma, nocardiosis and actinomycosis. This is my biggest criticism. Surely most authorities would now attribute erythrasma to corynebacterial infection rather than nocardia. The author does allow that mixed infections with those organisms may occur. The actinomycetes deserve more than a paragraph.

Chapter 5 is solid practical information on the isolation and identification of pathogenic fungi and includes details of the most useful common staining methods and culture media. I was particularly intrigued by the ingenious key by which one could logically work through various microscopic appearances to the identification of species of fungi in tissues.

Chapter 6 is a short appraisal of the usefulness or otherwise of serological methods in the diagnosis of candidosis and other systemic mycoses. This is quite balanced and its appearance in this edition is important. Although short, appropriate references for further reading are provided. I felt this chapter was inserted in the wrong position and interrupted the natural progression from Chapter 5 to Chapter 7 which together constitute approximately half the text and contain the real meat of the book. Chapter 7 is concerned with the cultural and microscopic identification of fungi and contains three of these delightful keys for sorting various groups of fungi, the species of *Aspergillus* and the yeasts respectively. In detail, I feel that the weight of evidence should have been sufficient to allow the inclusion of *T. Hendersonuli* in the list of agents infecting skin and nails. The author is also too strong in his condemnation of Wood's lamp as a diagnostic aid.

This chapter is very well illustrated with colour plates and line drawings and the text is packed with information set out with commendable clarity. Following this chapter only, one could achieve a high degree of competence in medical mycology without additional help. Many would disagree in details over the nomenclature in Table 1 listing the imperfect and perfect states of the dermatophytes but in the text the author deals with only those species which are common and of importance.

The handbook ends with short but useful chapters on mycotoxins, sterilisation of fungi (of doubtful value) and the chemotherapy of fungal infections. This last is up-to-date but not detailed. To conduct treatment more information would be necessary.

The book is well indexed and the bibliography is extensive including many of the more important references in the English language. In conclusion, this is a first class production. To my knowledge there has been nothing comparable published in English since the now out-of-date 'Manual of Medical Mycology'. I should have thought it was worthy of translation.

C.N.D. CRUICKSHANK

Review

Mycetoma

Mycetoma or 'Madura foot' commonly occurs in the tropics and subtropics; it is particularly prevalent in Africa, India, and Central and South America. The disease is occasionally seen in North America, but is almost unknown in Europe.

A mycetoma can be defined in strict mycological terms as a concretion of fungal hyphae which form a tumour or 'fungus ball' at any site within the animal body; the term can be correctly applied therefore to fungal balls that occupy cavities in the lung as in aspergilloma. However, mycetoma (which may occur in any part of the body in the skin and subcutaneous tissues) is a recognized disease and has a much more restricted clinical meaning though caused by a wide variety of organisms. These fungi either belong to the true or higher fungi (Eumycetes), or to that group of organisms, the Schizomycetes (Actinomycetes), which are intermediate between fungi and bacteria. For practical purposes these are best classified as bacteria. Two types of mycetoma can therefore be differentiated according to the causative organisms, and such a division is pertinent to the management of the patient.

(a) *Actinomycetoma*: this is caused by the aerobic Schizomycetes such as *Nocardia* and *Streptomyces*.

(b) *Eumycetoma*: this is caused by the Eumycetes (true fungi) such as *Madura* and *Cephalosporium*. Both the Eumycetoma and the Actinomycetoma present with similar clinical manifestations. The process usually affects the feet (Fig. 1) but may affect the legs, the arms and shoulders, or the head. Characteristically there is swelling of the affected area with multiple draining sinus tracts that exude pus containing granules of varying size and colour. Laboratory examination of these granules not only confirms the clinical diagnosis but may also determine the type of mycetoma, and thus the mode of treatment.

Mycetoma is usually seen in men in the third and fourth decades and minor injuries serve as a portal of entry for the infecting organisms which are saprophytes of soil and plants. Consequently the feet are the most frequently affected sites and this explains the synonym 'Madura foot'. The incubation period varies from one week to several months. The disease is not contagious from man to man, or from animals to man. Usually the condition begins as a painless nodule at the site of previous trauma; indeed the absence of pain is characteristic throughout the course of the complaint. This primary nodule softens and discharges pus. Subsequently the whole foot swells to such an extent as to cause the sole to assume a convex surface and the lesions extend deeply into subcutaneous tissue. Multiple secondary nodules develop and these suppurate and drain through multiple sinus tracts exuding a serous, sero-sanguinous, or purulent discharge in which pigmented granules may be found, especially during exacerbations. The colour of the granules varies according to the causative organism and so visual examination of the pus is the first step to the laboratory diagnosis. The infection remains localized and constitutional disturbance is rare but when it does occur it generally results from secondary bacterial infections of the open sinus tracts. In chronic cases the sinuses may extend deeply into the muscle and bone. Osteomyelitis may be marked and is readily detectable on X-ray which shows evidence of periostitis and cavities within the bone (Fig. 2).

Mycetoma of the abdomen may present difficulties diagnostically because of similarities to appendicitis. However, the correct diagnosis should be apparent when discharging sinuses are seen in the abdominal wall.



FIGURE 1. Mycetoma of foot, showing swelling of foot and multiple sinuses exuding pus.

DIAGNOSIS

At present the laboratory investigations are concerned with the exudate alone as serological tests are still only of academic value. However, serological tests may well in future become incorporated as a routine procedure for the specific identification of the aetiological agent. The mycological diagnosis is deduced from direct observation and from culture of the granules or pus.

(a) *Direct observation.* The presence and colour of the granules should be recorded and may well suggest the causative organism (see Table 1). Dark coloured granules are readily found but white to cream coloured granules can be difficult to recognize. Thick pus should be diluted with physiological saline and spread out in a suitable receptacle. Granules appear as discrete opaque bodies of roundish shape with a lobulated smooth shiny surface.

To enable microscopic examination, the granule should first be crushed and then mounted in a drop of 20% potassium hydroxide (KOH). A Gram stained smear should also be made. The hyphae from eumycetomas appear as broad septate branching filaments which frequently form chlamydo-spores in the peripheral region of the granule. They stain poorly but are generally considered to be Gram positive. Granules from actinomycetomas are composed of extremely fine hyphae (about 1 μ m wide) and these may be difficult to see in the KOH preparation. The stained film reveals delicate Gram positive branching filaments which may or may not end in a club shape in the peripheral region of the granule. Short bacillary elements may also be seen. Where no granules are found the pus should be examined by the same procedures.

The direct observations carried out on the exudate enable the type of mycetoma to be ascertained and so the correct method of therapy can be instituted in most instances before the results of culture are known.



FIGURE 2. X rays of mycetoma of foot, showing multiple lesions in metatarsals producing irregular expansion and destruction of bone. There is also soft tissue swelling and calcification.

TABLE 1. Species commonly associated with mycetoma

Type of mycetoma	Colour of granules	Species
Actinomycetoma	White to yellowish	<i>Nocardia asteroides</i>
		<i>Nocardia brasiliensis</i>
		<i>Nocardia caviae</i>
		<i>Streptomyces madurae</i>
	Yellow to brownish	<i>Streptomyces somaliensis</i>
	Bright red	<i>Streptomyces pelletieri</i>
	Black	<i>Streptomyces paraguayensis</i>
Eumycetoma	White to yellowish	<i>Monosporium apiospermum</i> (<i>Allescheria boydii</i>)
		<i>Cephalosporium falciforme</i>
		<i>Cephalosporium recifei</i>
		<i>Neostudina rosati</i>
	Yellow to brownish	<i>Phialophora jeanselmei</i>
		<i>Madurella mycetomi</i>
	Brown to black	<i>Madurella grisea</i>
	Black	<i>Leptosphaeria senegalensis</i> <i>Pyrenochaeta romeroi</i>

(b) *Culture*. In order to reveal the specific identity of the fungus or bacteria the specimen has to be cultured. Isolation of the causative organism will also allow drug sensitivities to be determined where applicable. Since the surface is likely to be heavily contaminated the granules should be washed several times in sterile physiological saline. They should then be crushed in saline and the resultant suspension provides a suitable inoculum for streaking onto agar media. In eumycetoma, a plate containing Sabouraud's medium with antibacterial antibiotics (though not with cycloheximide as this is inhibitory to a number of species causing mycetoma) should be inoculated and left for at least 3 weeks at 37°C, and one containing Littman ox-gall agar, should also be inoculated and kept for this time at 25–30°C before being discarded. Any bacteriological medium will support the growth of organisms causing actinomycetoma but beef heart infusion blood agar tends to be the preferred medium. A Petri dish containing this medium and one containing Sabouraud's agar without antibiotics should be incubated at 37°C. A duplicate set of plates should be incubated at 25–27°C. Again the plates should be retained for about 3 weeks. The organisms are identified according to specific characteristics and the more common isolates are listed in Table 1. Because the specimen is prone to contamination it may be necessary to isolate a species on several occasions before pathogenicity can be ascribed.

The clinical investigations should include X-ray of the affected area to determine the presence and extent of osteomyelitis; this may be a useful prognostic indication in the drug therapy of actinomycetoma.

TREATMENT

The correct treatment of mycetoma depends to a considerable extent on the causative organism. In general an actinomycetoma may be amenable to conservative treatment, whereas a eumycetoma will only be ameliorated by surgical intervention.

In a recent series Mahgoub (1976) reported a 60% 'cure rate' in actinomycetoma using either a combination of dapsone and streptomycin, or cotrimoxazole and streptomycin. Unfortunately the report does not mention the long term follow-up as patients on such drug regimes can show significant recurrence rates after the cessation of therapy.

In eumycetoma various antifungal agents have been tried but with little success; this is perhaps surprising as the fungi causing eumycetoma are low grade pathogens and so their eradication should be readily achieved by administration of a safe systemically active agent. However, as in the case of aspergilloma in lung tissue, it seems that therapeutic levels of the drug within the lesion are not attainable, probably due to poor blood supply to such areas. For this reason local injections of amphotericin B (Costello *et al.*, 1959; Mathews *et al.*, 1968) may prove worthy of evaluation. The newly discovered antifungal imidazoles may also prove useful in the future but the cost of such treatment will prevent widespread use in the 'Third World'.

In the meantime, for eumycetoma and for recurrent actinomycetoma, the most effective treatment is surgical excision of the affected area. This treatment may seem drastic, but ultimately the patient will be less crippled by a below-knee amputation and a prosthesis than by retention of a swollen diseased foot.

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Royal Infirmary and Mycology Unit,
Western General Hospital,
Edinburgh

R. StC. BARNETSON
L. J. R. MILNE

REFERENCES

- COSTELLO, M.J., DEFEO, C.P. & LITTMAN, M.L. (1959) Chromoblastomycosis treated with local infiltration of Amphotericin B solution. *Archives of Dermatology*, **79**, 184.
- MAHGOUB, E.S. (1976) Medical management of mycetoma. *Bulletin of the World Health Organisation*, **54**, 303.
- MATHEWS, R.S., BUCKLEY, C.E., NELSON, H.S., GRANT, A.J. & EDWARDS, W. (1968) Local chemotherapy of deepseated fungus infections; maduromycosis of the foot. *Clinical Research*, **16**, 48.

News and Notices

International Congress of Inflammation

An International Congress of Inflammation will be held in Bologna (Italy) from 31 October to 4 November 1978. Further information may be obtained from: Dr Emanuela Folco -FGL- Via Monte Napoleone, 23 20121 Milan, Italy.

APPOINTMENT

Kent Area Health Authority

Dr David Goldin has been appointed Consultant Dermatologist to Canterbury and Thanet. He was born and educated in Yorkshire and studied medicine at St Thomas' Hospital, London, graduating in 1969. After his house jobs he spent a year with the Zambia Flying Doctor Service. At Wycombe General Hospital he did joint medical and dermatological jobs at S.H.O. and Registrar level, and gained the M.R.C.P. in 1973. He was Senior Registrar at Addenbrooke's Hospital, Cambridge, for four years.

He held the commission of Surgeon Lieutenant in the Royal Naval Reserve, being attached mainly to the Royal Marines Reserve where he successfully completed commando training. His main professional interests lie in clinical dermatology and therapeutics. He has published a number of papers on physiology and disease states in African children, as well as case reports and papers of a general clinical interest in dermatology.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometer(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^a / _b
hour(s)	h	milliequivalent	mmol		

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Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on faint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 99, Number 2, August 1978

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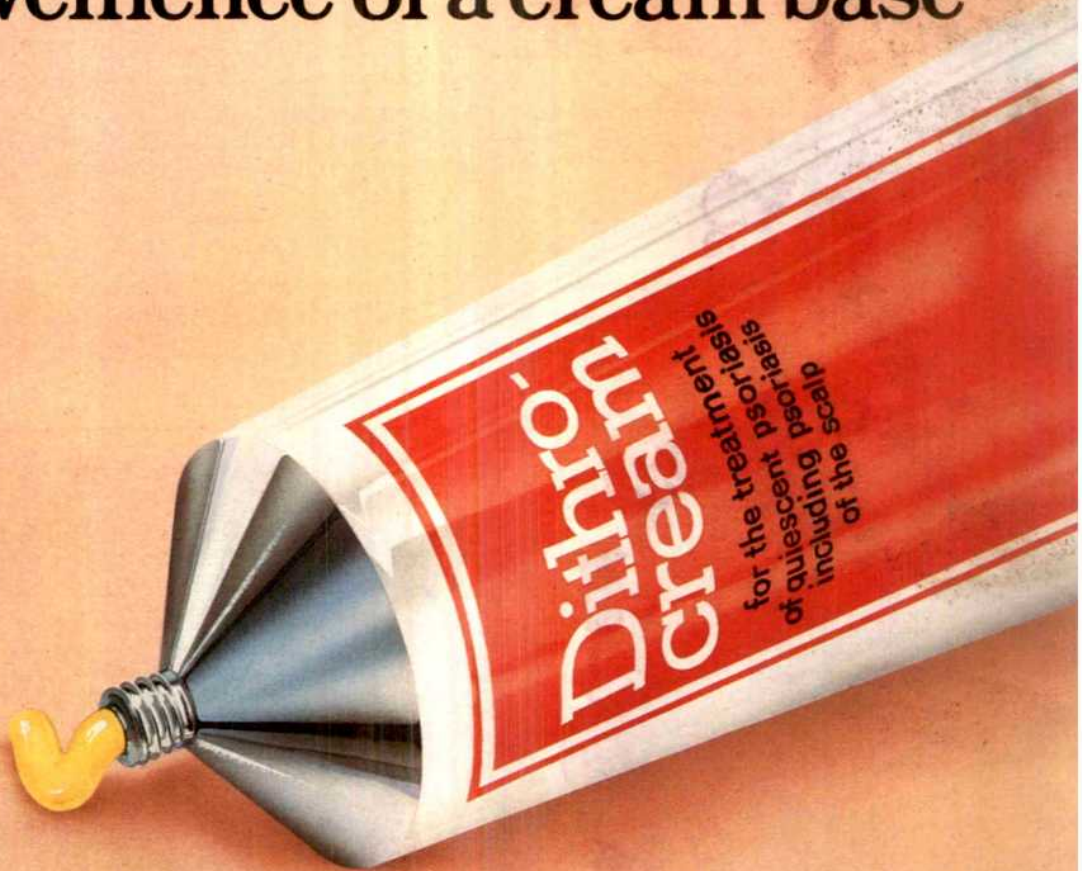
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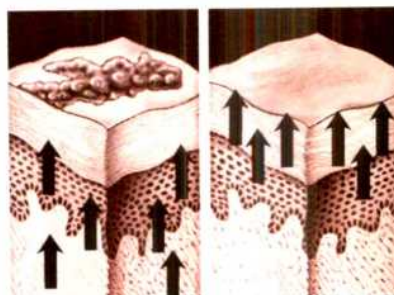
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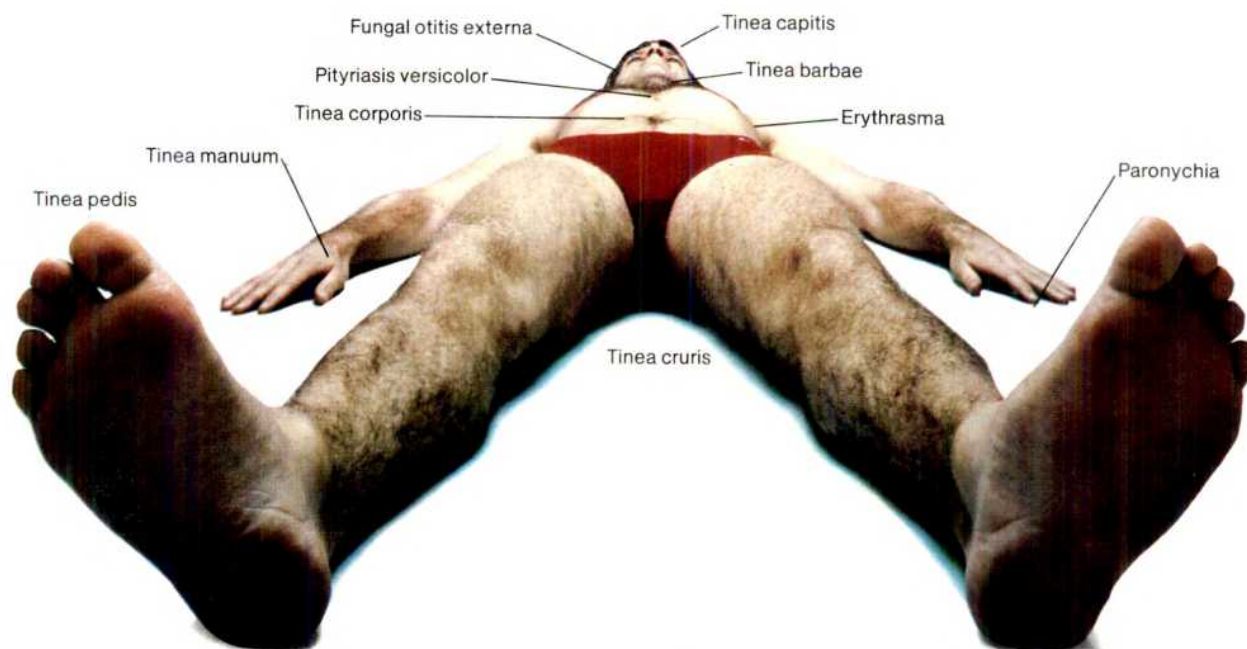
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Textbook of Dermatology

*Third Edition
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mild Eumovate offers greater efficacy than hydrocortisone, yet possesses a wide margin of safety.

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EUMOVATE: Apply up to four times a day until improvement occurs, when the frequency may be reduced.

BETNOVATE: Apply a small quantity two or three times daily until improvement occurs. Improvement may then be maintained by applying once a day, or less often.

DERMOVATE: Apply sparingly once or twice daily until improvement occurs. Treatment should not be continued for more than four weeks without review.

Side effects

General: With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.

Rarely, treatment of psoriasis with corticosteroids or its withdrawal is thought to have provoked the pustular form of the disease.

Precautions

Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy. With all corticosteroids, prolonged application to the face is undesirable.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

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Presentation Product Licence number

Eumovate:
Cream, Ointment 4/0233, 4/0254

Betnovate:
Cream, Ointment 4/5121, 4/5124
Lotion 4/5130

Dermovate:
Cream, Ointment 4/0219, 4/0220

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Trimovate

Cream (clobetasone butyrate 0.05% w/w, nystatin 100,000 units per gram and oxytetracycline 3.0% w/w as calcium oxytetracycline). Available in tubes of 25 grams. Ointment (clobetasone butyrate 0.05% w/w, nystatin 100,000 units per gram and chlortetracycline hydrochloride 3.0% w/w). Available in tubes of 25 grams.

Glaxo Leaders in topical steroid therapy

Further information on Trimovate (trade mark) is available from:
Glaxo Laboratories Ltd, Greenford, Middlesex UB6 0HE
Product licence numbers
Ointment — PL/0004/0265 Cream — PL/0004/0266

Prescribing information

Dosage and administration

Apply to the affected area up to four times a day.

Contra-indications

Viral (e.g., herpes simplex) and dermatophyte (tinea/ringworm) diseases of the skin, and tuberculous lesions.

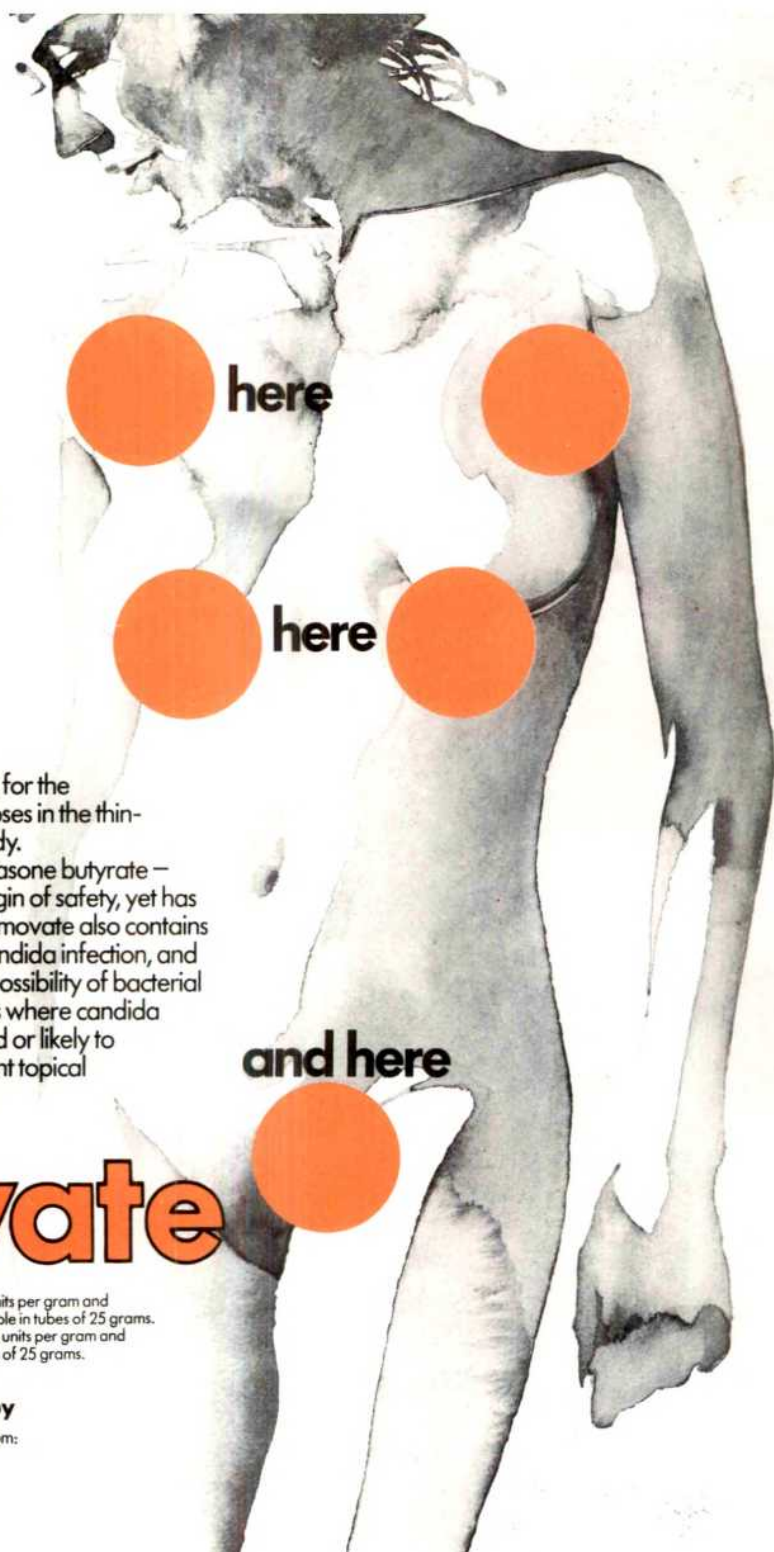
Precautions

In infants and children, long-term continuous topical steroid therapy should be avoided where possible, as adrenal suppression can occur even without occlusion. In infants, the napkin may act as an occlusive dressing and increase absorption. Any spread of

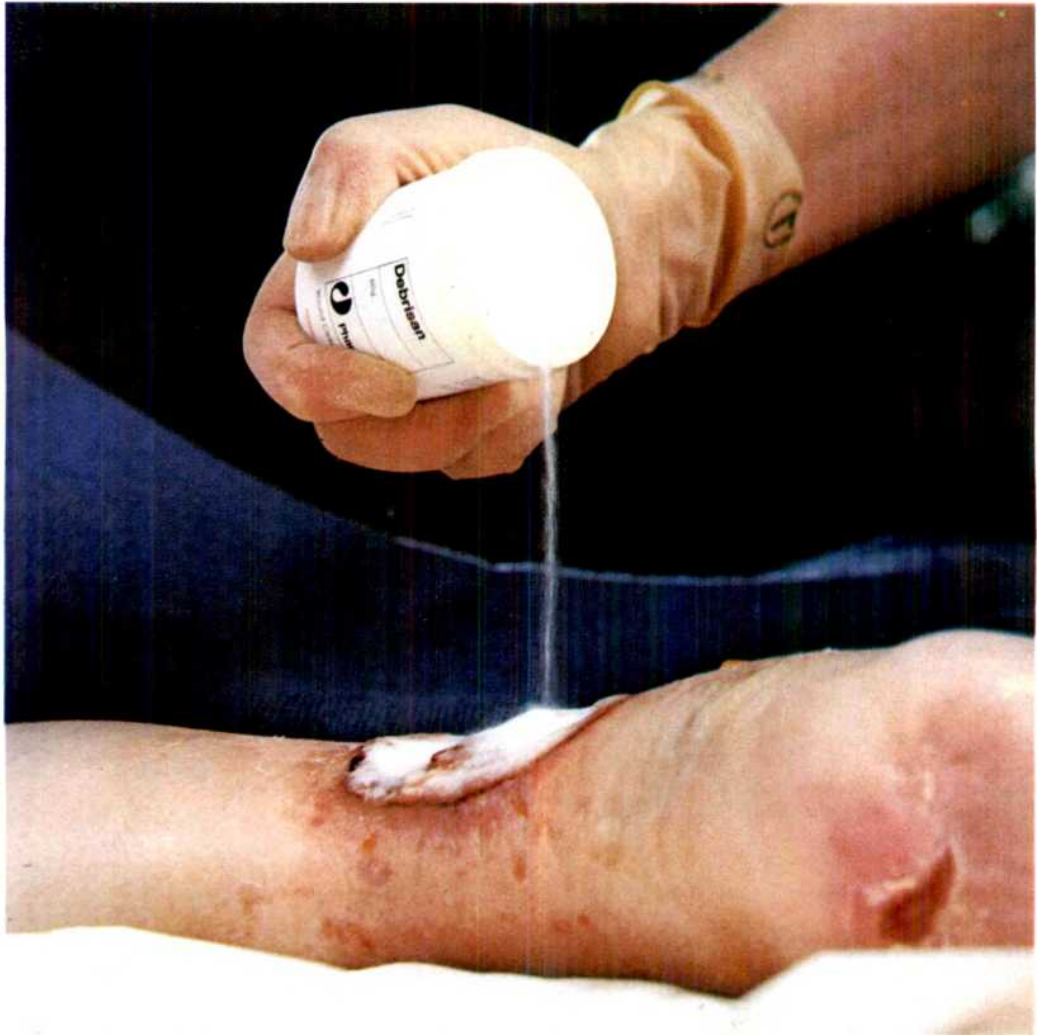
infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic chemotherapy. With all corticosteroids, prolonged application to the face is undesirable. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately. If large areas of the body were to be treated with Trimovate, it is possible that some patients would absorb sufficient steroid to cause transient adrenal depression despite the low degree of systemic activity associated with clobetasone butyrate. Local atrophic changes could possibly occur in situations where moisture increases absorption of clobetasone butyrate, but only after prolonged use.



Debrisan a major breakthrough in wound treatment



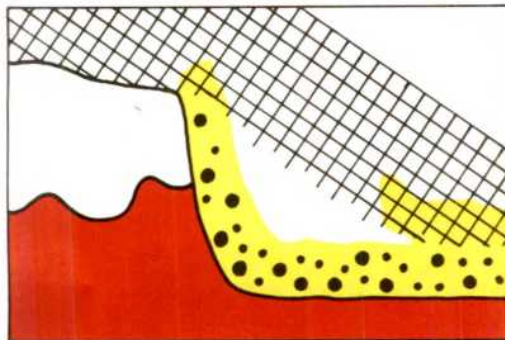
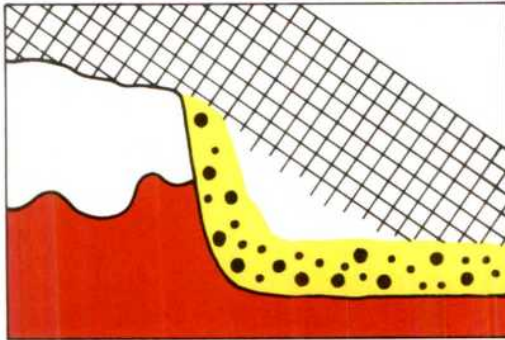
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Debrisan is available as a castor containing 60g of sterile dextranomer.

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Conventional dressing

Passive * partial *
sometimes painful



Top and bottom left

A conventional dressing may bridge the wound and hold a layer of pus against the wound surface.

Red: Oedema

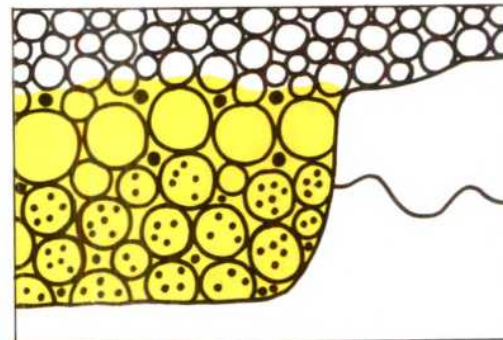
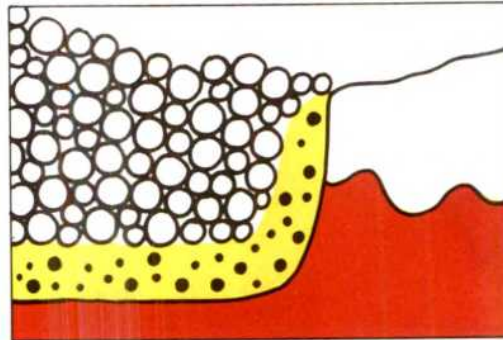
Yellow: Infected pus

Large black dots: Microorganisms and large molecules

Small black dots: Small molecules

Debrisan

active * continuous *
rapid relief of pain



Top right

Debrisan fills the wound achieving intimate contact with the wound surface.

Bottom right

Wound exudate is drawn into the Debrisan layer and away from the wound surface. Cleansing is continuous if Debrisan is replaced before saturation.

Debrisan consists of spherical beads of dextranomer 0.1-0.3mm in diameter which can suck up four times their own weight of moisture with considerable force. By continuous removal of bacteria and exudate by this means the wound is cleansed, often with the benefits of healing and decrease in malodour. Inflammation, oedema and crust formation are reduced. Grafts can often be made earlier through promotion of granulation tissue formation. Debrisan does not cause sensitisation and is thus of value in wounds such as leg ulcers where this is a major problem. Because of its mechanical mode of action Debrisan can be used to clear infected wounds prior to receipt of bacteriological reports. Early effective treatment with Debrisan can shorten the hospital stay to the benefit of all.

Indications

All kinds of secreting, suppurative wounds, such as post-operative wounds, leg ulcers, pressure sores, traumatic wounds, hand burns, suppurative ulcers, infected radio dermatitis, non-venereal penile ulcers.

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Pellicol contains Alkyl dimethyl benzyl ammonium halide dibromide 4.0% W/W; Benzalkonium chloride 0.5% W/W. PL 0173/0016



British Patent No. 1155258

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A.M. Johansen & B. Sørensen, Scand. J. Plast & Reconstr. Surg: 4:41-44:1970

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is unique in containing a
lipid phase analogous to human
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It is indicated where skin film
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is economical in use.
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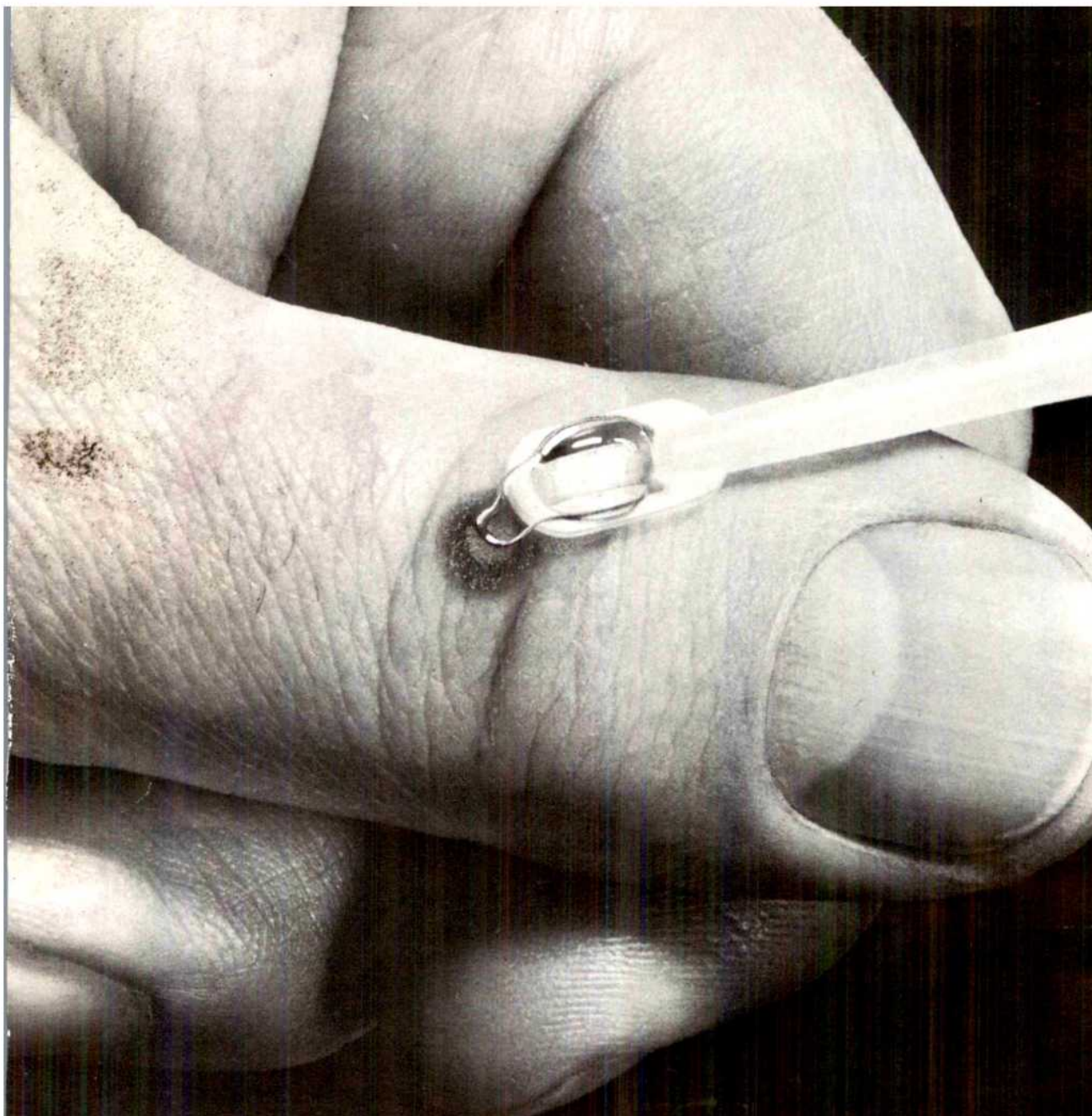


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Glutarol
For the
treatment
of warts

10ml
Active ingredient:
Glutaraldehyde 10%
PL 0173/0022

Possible role of connective tissue in epidermal neoplasia

E. MITRANI

Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff

Accepted for publication 3 March 1978

SUMMARY

Interactions between epidermal cells have been defined within a proposed mathematical model of mammalian skin. Testing the model in a computer suggests that in highly proliferating conditions of the epidermis competition for cell space in the basal layer may be sufficient to generate considerable forces in the papillary dermis. Data shown from human and pig epidermal hyperplasia indicate that basal cells are submitted to considerable lateral forces and that these and not dermal hyperplasia are the forces responsible for the increasingly folded dermo-epidermal junction. When the model was examined in condition of persistently high mitotic rate it was found that it could remain stable only if new connective tissue synthesis was not induced by the developing papillary tension. This complex and counterproductive relationship that may occur between epidermis and dermis and its possible role in the development of neoplasia are discussed.

A study of the possible interactions between cells which may play a role in epidermal homeostasis is presented here. An examination is made of how epidermis retains its mass both normally and after various disturbances and also how this mechanism may sometimes fail, as it does in neoplasia. Uncontrolled growth is examined in terms of defects in the system rather than in the individual units.

The basis of the model

The first assumption made here is that all the cells are born potentially equal, all having the possibility of multiplying by mitosis, or remaining in interphase or of dying through the ageing process. The sequence of stages through which a cell may pass is shown in Fig. 1. G1 has been subdivided into G1a and G1b. The mitotic pathway is not one of continuous progress and in particular at the stage G1b, a cell has a certain probability $p(m)$ of becoming mitotic, (Smith & Martin, 1973; Bullough & Mitrani, 1976). This probability does not depend on the time that the cell has been in this stage. The expected length of the stage G1b is considered equal to $1/p(m)$.

Quastler & Sherman (1959) noted that in the intestinal epithelium of the mouse, cells could not leave the crypt 'during or shortly after S phase' and that a period (of about 4 h) after mitosis had to

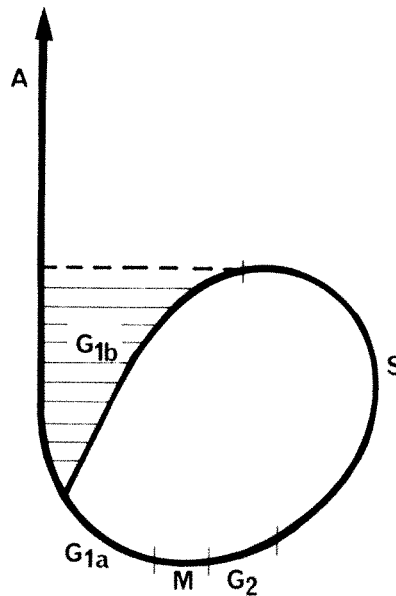


FIGURE 1. The cell life cycle. It is assumed that once a cell enters the mitotic pathway through the S phase it will complete mitosis after going through G₂ and M. G₂ in hyperplasia is assumed to have a short constant duration. G₁ has been subdivided into G_{1a} and G_{1b}. G_{1a} represents the fixed part of G_{1b} and is the minimum time, following M, before a cell can enter S. During the phase G_{1b} a cell can remain in that phase indefinitely, enter the mitotic pathway (S + G₂ + M) or proceed along the ageing pathway (A) to eventual death. Neither the mitotic pathway nor the ageing pathway, once entered upon, can be renounced. The reversible part of the ageing pathway is considered from the model point of view to be indistinct from the indeterminate stage G_{1b}.

pass before migration could occur. Marques-Pereira & Leblond, (1965) investigating autoradiographically the fate of the cells of stratified squamous epithelium of the oesophagus of young adult rats in serial sections concluded that basal cells appeared to be readily transferable from the basal layer only from the time they arise out of mitosis until they start synthesizing DNA prior to the next mitosis. This statement was shown to be essentially valid for hairless mouse dorsal epidermis by Iversen, Bjerkness & Devik (1968). Similarly Frankfurt (1967) found that labelled cells do not appear in the spinous layer of the squamous epithelium of the fore-stomach of the mouse until 32 h following the injection of ³H-thymidine. Since the length of the S phase was evaluated at 7 h and of G₂ at 3 h it is unlikely that M would have taken 22 h and therefore not only did cells not leave the basal layer during S and G₂ and M but a further time lag (G_{1a}) elapsed before cells started to appear in higher layers. Thus in the model it is postulated that there are two grades of basal grip; one is strong and comprises the stages S + G₂ + M + G_{1a}, during which a cell never lets go, while the other in G_{1b} is comparatively weak is perhaps viscous in nature (Leun, Lowe & Beerens, 1974) and during this phase cell extrusion is pressure and time dependent. It is assumed that mitotic activity is controlled by a chalone gradient (Bullough, 1975) and since in most epidermis mitosis is largely confined to the basal layer and perhaps to those layers immediately above it (Penneys *et al.*, 1970) it is assumed that chalone effectiveness is at its minimum basally and at its maximum distally (see Bjerkness & Iversen, 1974). In essence the model assumes that the lower the layer (i.e. nearer to the dermis) the higher p(m), the probability of a cell in that layer to enter mitosis. It is assumed that if a cell spends more

than a certain time T_d in G1b, without being directed into the mitotic pathway, it will enter the ageing pathway and progress irreversibly towards keratinization and death.

Some speculation can be found in the literature regarding the possibility of cells actively migrating from the basal layer in normal steady state situations (Winter, 1964; Etoh, Taguchi & Tabachnick, 1975). There is indeed evidence for active migration in other circumstances, such as wound healing *in vivo* (for a detailed description, see Winter, 1972) or 'epiboly' formation *in vitro* (Marks & Nishikawa, 1973) but only in the recent work of Etoh *et al.* (1975) has an attempt been made to demonstrate active distal cell migration in conditions where ideally only mitosis has been suppressed. The validity of Etoh *et al.*'s (1975) experimental approach can be questioned; however, assuming their findings are correct, a re-analysis of their data (Mitrani, 1976) would show that if active migration is the driving force, the kinetic parameters reported by Etoh's group, once mitosis is suppressed, would impose a depopulation value considerably higher than the one observed experimentally. In the absence of any strong evidence for active migration taking part in normally renewing epidermis the present model is based on the postulate that in normal mammalian epidermis G1b cells are *forced* out of the basal layer by the stress generated by nearby dividing cells. This concept was originally proposed by Bizzozero (1888) and was later particularly supported by the work of Leblond, Grulich & Pereira, (1964) on oesophageal epithelium. Normally for every new cell that is produced another is extruded to become post-mitotic and eventually to die.

OBSERVATIONS

In considering cellular interactions, it became clear that forces between cells may play a critical role in the regulation of the epidermal cell mass and it was found necessary to obtain further information on this subject. In particular, it was necessary to study the grip of the basal cells on the dermal surface, the potential forces generated in the basal layer, and the significance of baseline folding in normal and hyperplastic conditions.

The shape of the nuclei of basal cells will be considered as an indicator of the forces acting upon the keratinocytes. It will be assumed that the nuclear membrane is elastic and there are no constraints to it being deformed as a function to the forces imposed on it by the cytoplasmic content. If these forces are equal in all directions the nucleus should be spherical, if they are higher in the horizontal plane, the cell should be columnar and the nuclear ratio (vertical diameter of the nucleus over horizontal diameter of the nucleus) should be higher than one. Orientations of the section should not affect the nuclear ratio since any section through a spherical cell will project that cell as a circle, whilst a columnar cell will only be projected as a circle if the section is cut perpendicular to the long axis. Fixation of the tissue for histological examination probably has an effect on the shape and volume of the cells and for this reason it is important to confirm reproducibility of results. Undifferentiation does not seem to be a necessary condition for columnarity since in hyperplastic mouse ear, basal cells maintain a nuclear ratio of one, whilst in pig or human epidermal hyperplasia the nuclear ratio is considerably greater (see below). Conversely during late catagen for instance, basal cells with a nuclear ratio greater than one, can be found in the middle third of the hair follicle of the pig where no mitoses are known to take place (Winter, 1966).

Both normally occurring and experimentally induced hyperplasia in man and pig were examined and the nuclear ratio for each individual basal cell was recorded. The baseline ratio, i.e. the degree of folding of the baseline, was also recorded. Table 1 shows the means and standard errors obtained for each of the situations considered. N represents the number of cases in each situation; at least 100 cells were counted for each case. The pattern for mouse and guinea-pig epidermis (data not shown) is somewhat different; the increased rate of production of cells in hyperplasia is offset by a

TABLE 1. Dynamic parameters of normal and hyperplastic epidermis

Species	Condition of epidermis	N	Baseline length per mm surface length	Estimated birth rate (cells/h/cell)	Nuclear height/width ratio	Epidermal thickness μm
Human	Hypoplasia					
	Induced*	7	1.02 ± 0.04	—	1.41 ± 0.13	—
	Senile	3	1.02 ± 0.02	—	1.28 ± 0.29	—
	Total	10	1.02 ± 0.01	—	1.37 ± 0.12	43.6
	Normal					
	Body	15	1.28 ± 0.09	$0.0036 \ddagger$	1.66 ± 0.10	67.2
	Hyperplasia					
	Lichenification	7	2.48 ± 0.28	—	2.94 ± 0.26	—
	Psoriasis	13	2.77 ± 0.33	$0.0130 \S$	2.62 ± 0.13	190.1
	Induced†	4	2.81 ± 0.12	—	2.27 ± 0.10	—
Pig	Total	24	2.69 ± 0.19	—	2.66 ± 0.11	193.1
	Normal	12	1.31	$0.006 \P$	1.90	52.5
	Hyperplasia					
	Mild	12	1.26	—	1.93	—
	Strong	12	3.10	$0.090 \P$	3.40	141.2

* By glucocorticoid treatment.

† By irradiation.

‡ Allegra & Depanfilis (1974). The correction factor of 1.4 has been ignored.

§ Duffil *et al.* (1976).

¶ Data derived from Winter (1972). Based on the assumption that in pig epidermis the rate of metaphase accumulation is constant for at least 4 h and does not significantly differ from the slope of the line joining the origin and the metaphase index 4 h after colchicine administration.

sufficient number of cells that divide vertically and clearly this number does not interfere with the allocation of cell space in the basal layer. This process has been described elsewhere (Bullough & Mitrani, 1978). In human and pig hyperplasia there are two possible explanations for the observed increase in folding of the baseline and for the increased nuclear ratio of the basal cell. The first suggests that the primary cause of the increase in folding is the upthrusting of the oedematous hyperplastic capillary loops in the upper dermis which compress the intervening epidermal pegs as a result of dermal hyperplasia. There are two main arguments against this interpretation:

(a) horizontal sections, made parallel to the skin surface through the epidermal pegs, show that only the basal cells are compressed into a columnar shape with their long axes perpendicular to the baseline while the non-basal cells in the centres of the pegs are not compressed (see Fig. 2).

(b) the basal epidermal cells that are not within the pegs but are on the crests of the folds are not found to be extended.

Furthermore, an intermediate state can be found at the edge of a psoriatic plaque in which the baseline is not sufficiently folded but in which the basal cells (as measured by the nuclear ratio) are found to be more compressed than normal (Mitrani, 1976).

The second explanation for the folded baseline in a hyperplastic system may be that the increased number of mitoses creates an abnormal demand for places in the basal layer which cannot be satisfied by the available cells to be extruded. As more cells are being produced overcrowding occurs and this

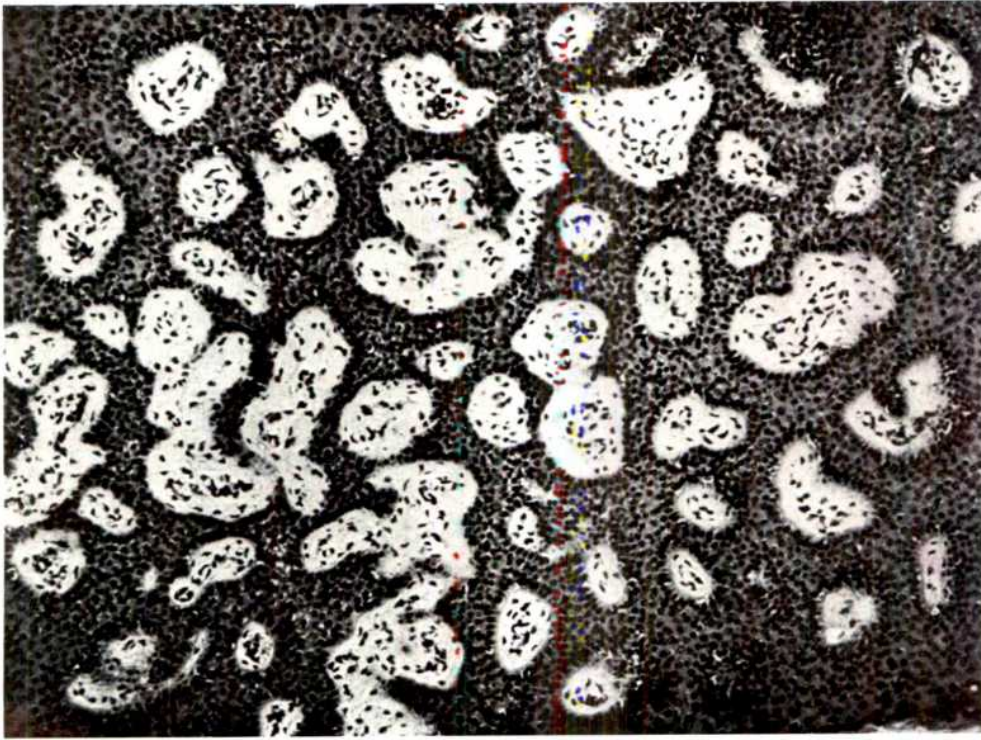


FIGURE 2. Horizontal section of pig skin through the dermal papillae to illustrate that if there is increased lateral stress between the basal cells in increasingly folded epidermis it is not created by the upthrusting dermal hyperplasia which may press on the intercapillary epidermal ridges. If it were due to dermal hyperplasia all cells within a ridge would be equally compressed; this is contrary to observations. It is clearly seen that only those epidermal cells in the periphery of the epidermal ridges are compressed indicating that the responsible forces must arise within the epidermal basal cells. (Photo by G. Winter.)

may bring about considerable horizontal stresses between the cells. This horizontal stress component will act on two elements; the basal cells themselves and the underlying tissue. As the cells are squeezed against each other the dermis will tend to expand in the horizontal plane. As the connective tissue system tries to expand, since the overall area is fixed, the system will be forced to buckle (see Fig. 3), but will nonetheless remain under tension. The explanation for the 'palisade' arrangement of the basal cells as disclosed in a horizontal section through a dermal peg (see Fig. 2) is now apparent.

It is therefore postulated that there are two major forces operating on the epidermal basal cells—the global force F_n , that may be generated by an excess of cells per unit area, and the local force F_m which is caused by a dividing cell and which has a direct effect on the detachment of surrounding Glb cells. We have assumed that the time taken for a basal Glb cell to let go should be directly related to a viscosity constant η and inversely related to the forces (F_n and F_m) acting on the cell. A similar relationship has been derived for dermal-epidermal adherence in a macroscopic system in epidermal suction pressure induced blisters (Leun *et al.*, 1974). Repercussions on the model of the type of functions adopted for F_n and F_m have been examined (Mitrani, 1976) and will not be discussed here. In the present model at each time interval, a cell in Glb will add to its history both the

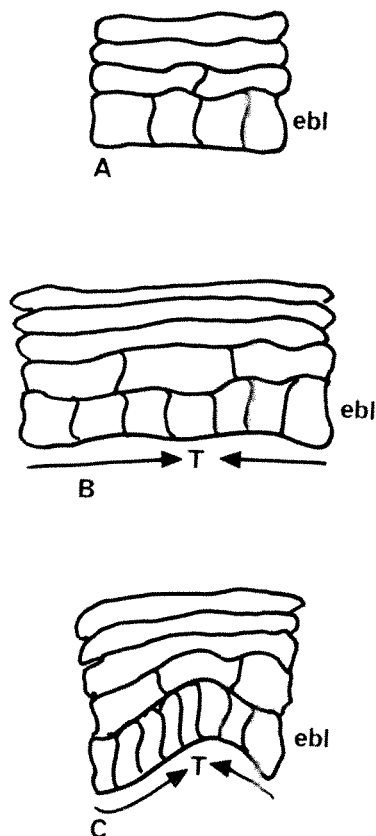


FIGURE 3. Diagrammatic explanation of the increase in folding for a given segment of skin. The normal situation is depicted in A. Ideally, as more cells tend to remain basal during an increase in the birth rate, the segment would tend to expand in the horizontal direction and cause tension in the dermis (B). However since the overall area is fixed, the segment in order to expand is forced to fold (C). The dermis will still remain in tension (ebl = epidermal basal layer, T = tension).

detachment fraction due to the global horizontal stress (F_n) and the detachment fraction which may be caused by the force F_m generated by a nearby dividing cell. Whenever a basal cell divides it will tend to do so within that layer and will tend to extrude a nearby Glb basal cell. However, if none of the nearest neighbours of a dividing cell are in Glb, the cell will divide vertically with one of the daughter cells being immediately located out of the basal layer; alternatively if one or more nearest neighbour cells are in Glb but the force F_m , generated by the basal mitosis, is not enough to extrude any Glb cell within a given neighbourhood, all cells in that neighbourhood will remain basal and will thus contribute to the crowding of the basal layer. A detailed description of the model and of its behaviour under steady state conditions is given elsewhere (Mitrani, 1978).

EXPERIMENTS IN MACHINA

Normally Glb occupies a significant fraction of the cell cycle. However, in hyperplasia the considerable decrease in cycle duration is almost totally due to shortening of the phase G1 (Baserga & Wiebel, 1968). The graph shown in Fig. 4 expresses the probability of a dividing cell not finding a Glb cell

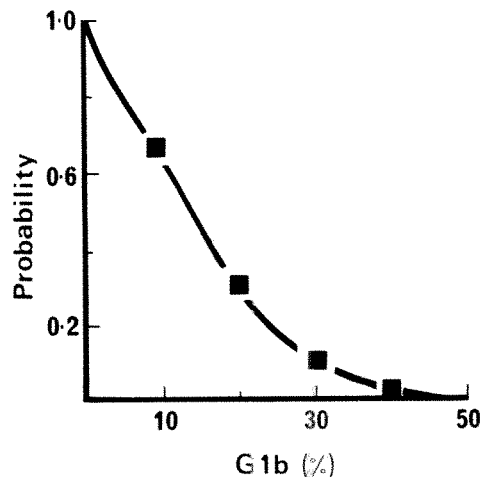


FIGURE 4. Relation between the probability of not finding a basal cell in G1b at any given time, and the duration of G1b expressed as a percentage of the total cycle duration. ($G1a = 13$ h, $S + G2 + M = 13$ h).

to be extruded, as a function of the relative duration of G1b (see legend for details). If for instance the G1b duration falls from the normal value of 80–90% to a value of only 20% of the total cycle duration, the figure indicates that out of every 10 basal mitoses, on average four will not find suitable cells to be extruded and consequently alternative ways will have to be found to allocate 40% of all newly formed cells. If all newly born cells were to remain basal there would be an increased number of cells per unit area and a consequent increase of folding of the baseline would result. The argument presented above would remain essentially the same if instead of a homogeneous basal population constituted only by cycling cells (basal growth fraction = 1), it is assumed that less than 50% of the basal cells are non cycling cells (basal growth fraction 0.5) (see Gelfant, 1977). In psoriasis, however, it is unlikely that few if any basal cells belong to the non cycling population. A value of 57 h for the cell cycle time in psoriasis has been determined by three different methods by Duffil, Wright & Shuster, (1976), assuming a growth fraction of 1. However, if say only 50% of the basal cells were in fact cycling in psoriasis these cells would have had to divide in half the stated time i.e. in 28.5 h in order to be consistent with the birth rate found. Such short cell cycle time for the cycling cells in psoriasis is unlikely since Duffil *et al.* (1976) found no evidence of a second peak on their fraction labelled mitosis curve over a period of 45 h after pulse labelling.

Role of the dermis

From the above findings, it is evident that as the probability of mitosis increases, stresses may develop in the basal layer and so force the underlying dermis into tension as is indeed observed in the increased folded baseline of some hyperplastic conditions. In those shown in Table 1 it is clear that there is an increase in the number of cells per unit area and that some of the stress thus generated must be transmitted to the folding baseline.

Figs 5 and 6 illustrate the behaviour of the model when it was operated under a low mitotic rate ($p(m) = 1/100$ h) during 700 h after which the mitotic rate was increased ($p(m) = 1/30$ h) to such an extent that folding set in. The system does reach a new equilibrium point even when the high mitotic rate remains. Cell interactions under the conditions that have been described are sufficient to take the system to a new equilibrium point. The change in mitotic rate is absorbed by folding,

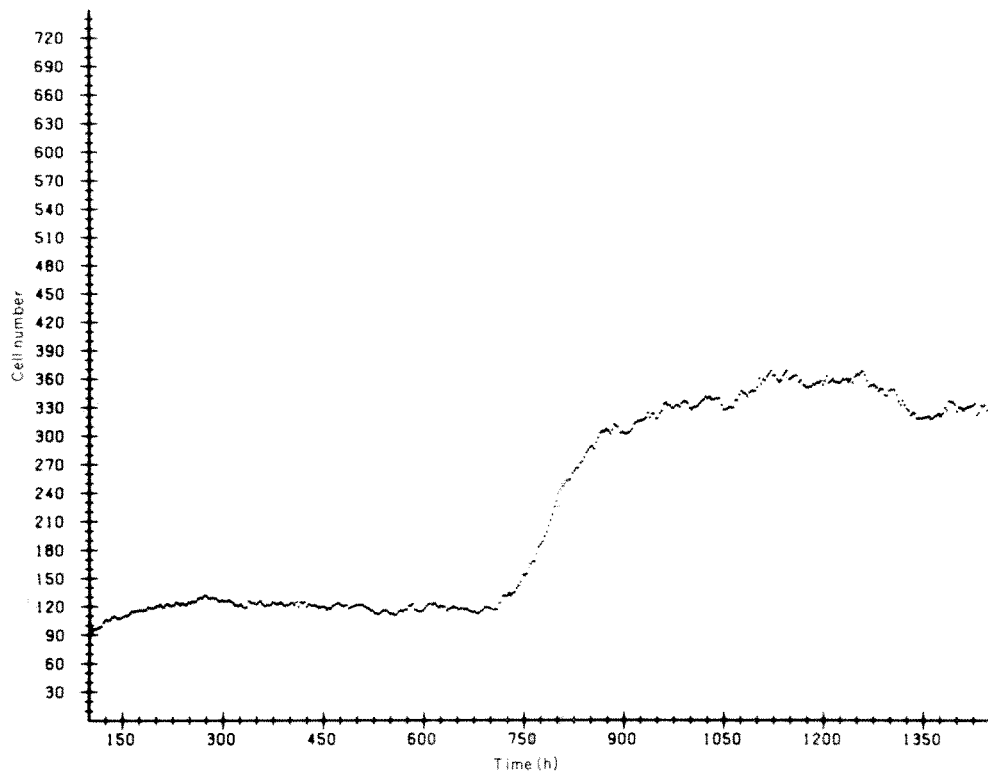


FIGURE 5. Total number of epidermal cells per unit length before and after a perturbation at time = 700 h consisting of a decrease in the expected length of G_{1b} from 100 to 30 h. Note how a new equilibrium position is reached in hyperplasia. Other parameters used were: viscosity constant (ck) = 30 (arbitrary units), G_{1a} = 10 h, $S + M + G_2$ = 10 h, and $F_n = (N_8 - N_9)/N_9$. N_9 is the number of cells per unit area in normal conditions and N_8 is the number of cells per unit area under folding conditions.

basal stress remains high, the number of cells per unit skin area increases greatly, but once again a balance between cell production and cell loss is achieved. However, if as has been suggested, there is involvement of the dermis by way of new collagen synthesis, then the balance of cells produced to cells extruded will be upset and no equilibrium point can be reached as shown in Fig. 7 (for explanation see legend). New collagen tissue formation will only relieve the stress temporarily; soon the epidermis will be in a situation similar to the one prevailing before synthesis had started, except that it would have induced a given amount of dermal growth. A self expanding system will emerge.

DISCUSSION

The conclusions are as follows:

- (1) The potential horizontal stress in the basal epidermal layer can be quantified in terms of the ratio G_{1b} duration to total cycle duration and a 'critical mitotic region' can be established.
- (2) The increased baseline folding in hyperplasia results from the horizontal forces developing between the basal cells and not from dermal oedema and dermal hyperplasia.
- (3) Stability cannot be achieved in chronic epidermal hyperplasia if the dermis responds by new connective tissue synthesis.

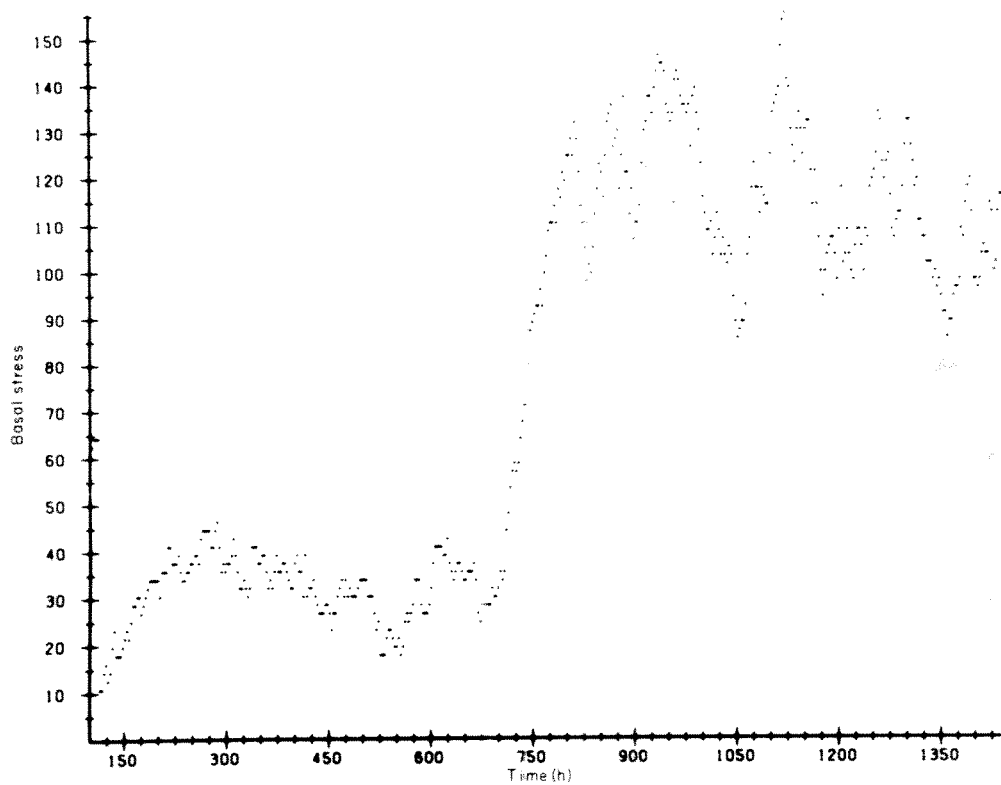


FIGURE 6. Time course of the horizontal basal stress (F_n) for the experiment described in Fig. 5. There is a marked increase in the magnitude of F_n after the decrease in the length of G1b from 100 to 30 h indicating an increase in crowding and/or folding of the basal layer.

A previous analysis (Mitrani, 1976) has indicated that the stability of the system is fairly insensitive to both quantitative and qualitative fluctuations in most of its components. Two conditions, though, are sufficient for instability to develop: the mitotic rate, for whatever reason, must be high enough to generate horizontal stresses in the basal layer and a sufficient amount of new connective tissue synthesis must follow. These circumstances will lead to new tissue formation. It seems that even though some workers have recognized the relevance of connective tissue synthesis in the development of an epidermal tumour, the way in which the epidermis in its growing process carries with it the dermis has remained virtually unexplored (Abercrombie, 1957). In fact the well established changes that occur in the dermis in the early stages of epidermal neoplasia are generally associated with connective tissue breakdown rather than connective tissue synthesis. The relation of mechanical stress to collagen formation is well established (Harkness, Harkness & Moralee, 1955). For instance it is noticeable in pregnancy that both skin and uterus form extra connective tissue, and this is evidently because of tension since humoral stimulation alone induces relatively little collagen formation (Harkness *et al.*, 1955). Similarly, bone submitted to chronic forces reaches a new equilibrium with collagen formation in the lines of maximum stress (Vaughan, 1970). Most workers have looked at the changes in collagen content rather than at the changes in the rate of collagen biosynthesis during experimental carcinogenesis (see review by Mazzucco, 1972). Nevertheless an early alteration during skin carcinogenesis consists of an increase in the dermal cells present and an alteration in the texture of the relatively coarse refractile

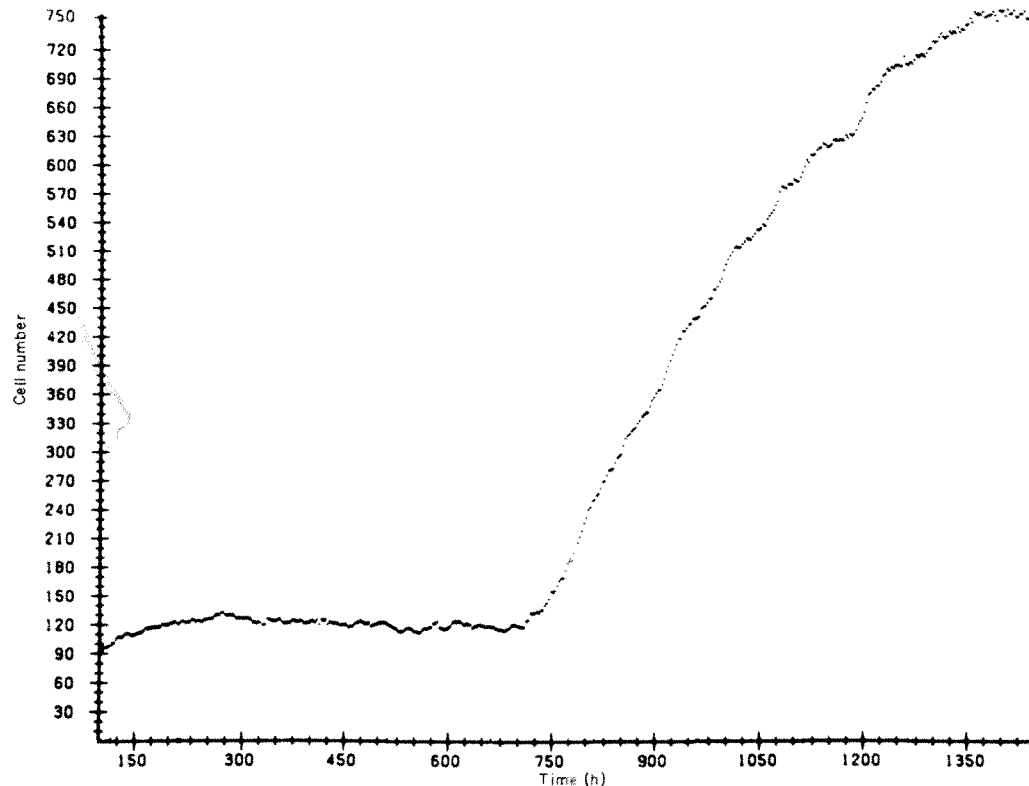


FIGURE 7. Total number of cells per unit area before and after a perturbation at time = 700 h consisting of a decrease in the expected length of G_{ib} from 100 to 30 h. The figure depicts an identical situation as that described in Fig. 5 except that for this experiment new connective tissue is allowed to form by an amount DL proportional to the basal stress present i.e. $DL = (N_8 - N_9)/2$. This system is no longer stable. (The apparent beginning of a second plateau (1350 h) is an implementation artefact representing a saturated condition of the actual computer model.)

bundles of collagen so that they become fine and non-refractile. This change spreads downwards and eventually the whole of the dermis acquires a fibrillary appearance as the normal fibres are replaced by thinner ones (Orr, 1938; Tarin, 1972). This series of events is clearly not a result of decreased collagen synthesis. It is often thought that epidermal tumours break their way through the dermis and for this reason attention has been focused in the role of collagenase. However, in a recent review of the role of collagenase in tumour invasion Strauch (1972) 'considers it to be proven that the first phase of carcinogenesis is associated with an increase in general tissue metabolism and of collagen metabolism in particular'. More recently Bauer *et al.* (1977) were unable to find any signs of collagenase among tumour epidermal cells *per se* in basal cell carcinomata using immunocytochemical techniques, although they were able to detect a slightly higher level of immunoreactive collagenase (iHSC) quantified by radioimmunoassay. They concluded that in any given specimen the level of iHSC appears to be a reflection of 'the relative amount of connective tissue in the sample'. The role of collagen synthesis on experimental epidermal tumours can best be illustrated by the work of Seilern-Aspang, Mazzucco & Christian (1969). These workers have noticed the absence of collagen decrease and the impossibility of inducing carcinomata in adult mouse tail *in situ* after successive application of chemical carcinogens. Appropriate controls excluded the possibility that differences in the pene-

tration of carcinogen were the responsible factors. It is interesting, however, that the level of collagen does not seem to be altered in adult mouse tail skin by carcinogen treatment. Mazzucco (1972) established that this fact may have a connection with the failure of the method to induce carcinomas in adult mouse tail since he was able to induce carcinomas in the tails of very young mice which had not yet reached the final collagen level and degree of crosslinking.

More recent work emphasized further the state of the stroma in tumour pathogenesis; Hodges, Hickes & Spacey (1977) have examined the carcinogen treated rat bladder *in vivo* and *in vitro* and have concluded that normal urothelial cell differentiation can be shown to be directed by stromal influences and that N-methyl-N-nitrosourea may elicit the appearance of a new epithelial behaviour by causing a derangement of stromal function. Cooper & Pinkus (1977) have similarly shown that the basal cells of a rat basal cell carcinoma lose their undifferentiated condition when they lose contact with the original dermal connective tissue. Chaudhuri *et al.* (1974) have examined the relationship among dysplasia carcinoma *in situ*, and invasive carcinoma of the human uterine cervix by utilizing the *in vitro* growth properties in epithelial cells and stromal fibroblasts in 25 clinical cases. Little differences were apparent between the epithelial cells; however, the stromal fibroblasts underlying a dysplastic area showed a predominantly uniform growth pattern, while the fibroblasts from the stroma underlying carcinoma *in situ* and invasive carcinoma exhibited a predominantly disorganized growth pattern with criss-crossing, and grew at about twice the rate of fibroblasts from regions of dysplasia. Only a small minority of oncologists have considered that carcinogenesis might entail intereactions between tissues (Burch, 1977).

Clearly collagen biosynthesis plays an important role in epithelial neoplasia. Experiments are now in progress to examine the state of collagen biosynthesis underneath benign, premalignant and malignant epidermal lesions by immunofluorescent techniques employing a specific dermal procollagen antibody.

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REFERENCES

- ABERCROMBIE, M. (1957) Localised formation of new tissue in an adult mammal. *Symposium Society of Experimental Biology*, **11**, 235.
- ALLEGRA, F. & DEPANFILIS, G. (1974) An *in vivo* method of studying the kinetics of cell proliferation in normal human epidermis. *Acta dermato-venereologica*, **54**, 87.
- BAUER, E.A., GORDON, J.M., REDDICK, M.E. & EISEN, A.Z. (1977) Quantitation and immunocytochemical localization of human skin collagenase in basal cell carcinoma. *Journal of Investigative Dermatology*, **69**, 363.
- BASERGA, R. & WIEBEL, F. (1969) The cell cycle of mammalian cells *International Review of Experimental Pathology*, **7**, 1.
- BIZZOZERO, G. (1888) Über die Regeneration der Elemente der Schilddrüsen und des Epithels des Magendarmkanals. *Anatomischer Anzeigen Cent.* **3**, 781.
- BJERKNES, R. & IVERSEN, O.H. (1974) 'Antichalone' a theoretical treatment of the possible role of antichalone in the growth control system. *Acta Pathologica et microbiologica Scandinavica*, suppl. **248**, 33.
- BULLOUGH, W.S. (1975) Mitotic control in adult mammalian tissues. *Biological Reviews*, **50**, 99.
- BULLOUGH, W.S. & MITRANI, E. (1976) An analysis of the epidermal chalone control mechanism. In: *Chalone* (Ed. by J.C. Houck), p. 7. Elsevier, North Holland.
- BULLOUGH, W.S. & MITRANI, E. (1978). The significance of vertical mitosis in epidermis. *British Journal of Dermatology*, **99**, in press.

- BURCH, P.R.J. (1976) *The Biology of Cancer. A New Approach*. MTP Press, Lancaster.
- CHADHURI, S., KOPROWSKA, I., PUTONG, P.B. & TOWNSEND, D.E.R. (1974) Human *in vitro* system of the detection of uterine cervical preinvasive carcinoma. *Cancer Research*, **34**, 1335.
- COOPER, M. & PINKUS, H. (1977) Intrauterine transplantation of rat basal cell carcinoma as a model for reversion of malignant to benign growth. *Cancer Research*, **39**, 2544.
- DUFFIL, M., WRIGHT, N.L. & SHUSTER, S. (1976) The cell proliferation kinetics of psoriasis by three *in vivo* techniques. *British Journal of Dermatology*, **94**, 355.
- ETOH, H., TAGUCHI, Y.H. & TABACHNICK, J. (1975) Movement of beta-irradiated epidermal basal cells to the spinous-granular layers in the absence of cell division. *Journal of Investigative Dermatology*, **64**, 431.
- FRANKFURT, O.S. (1967) Cell proliferation and differentiation in the squamous epithelium of the fore-stomach of the mouse. *Experimental Cell Research*, **46**, 603.
- GELFANT, S. (1977) A new concept of tissue and tumour cell proliferation. *Cancer Research*, **37**, 3845.
- HARKNESS, M.L.R., HARKNESS, K.D. & MORALEE, B.E. (1955) Effect of hormones on the collagen content of the rats uterus. *Journal of Physiology*, **128**, 16.
- HODGES, G.M., HICKS, M.R. & SPACEY, G.D. (1977) Epithelial-stromal interactions in normal and chemical carcinogen treated adult bladder. *Cancer Research*, **37**, 3720.
- IVERSEN, O.H., BJERKNES, R. & DEVIK, F. (1968) Kinetics of cell renewal cell migration and cell loss in the hairless mouse dorsal epidermis. *Cell and Tissue Kinetics*, **1**, 351.
- LEBLOND, C.R., GRUELICK, R.C. & PEREIRA, J.P.M. (1964) Relationship of cell formation and cell migration in the renewal of stratified squamous epithelia. *Advances in the Biology of Skin*, **5**, 39.
- LEUN, VAN DER, T.C., LOWE, L.B., Jr & BEERENS, E.C.J. (1974) The influence of skin temperature on dermal-epidermal adherence, evidence compatible with a highly viscous band. *Journal of Investigative Dermatology*, **62**, 42.
- MARKS, R. & NISHIKAWA, T. (1973) Active epidermal movement in human skin *in vitro*. *British Journal of Dermatology*, **88**, 245.
- MARQUES-PEREIRA, J.P. & LEBLOND, C.P. (1965) Mitosis and differentiation in the stratified squamous epithelium of the rat esophagus. *American Journal of Anatomy*, **117**, 73.
- MAZZUCO, K. (1972) The role of collagen in tissue interaction during carcinogenesis in mouse skin. In: *Tissue Interactions in Carcinogenesis* (Ed. by D.Tarin), p. 377. Academic Press, London.
- MITRANI, E. (1976) A theoretical analysis of cellular interactions leading to epidermal homeostasis in the adult mammal. Ph.D. Thesis, London University.
- MITRANI, E. (1978) A computer model for epidermal cellular interactions (submitted for publication).
- ORR, J.W. (1938) The changes antecedent to tumour formation during the treatment of mouse skin with carcinogenic hydrocarbons. *Journal of Pathology and Bacteriology*, **46**, 495.
- PERINGER, E., SEILERN-ASPANG, F. & MAZZUCO, K. (1970) Unterschied in der Abbau-Geschwindigkeit durch Kollagenase zwischen Schwanz- und Rücken-haut Kollagen von Mäusen in Beziehung zu der Induzierbarkeit von Karzinomen. *Archiv für Geschwulst Forschung*, **35**, 257.
- PENNEYS, N.S., FULTON, J.E., WINSTEIN, G.D. & FROST, P. (1970) Location of proliferating cells in human epidermis. *Archives of Dermatology*, **101**, 323.
- QUASTLER, H.R. & SHERMAN, F.G. (1959) Cell population kinetics in the intestinal epithelium of the mouse. *Experimental Cell Research*, **17**, 420.
- SEILERN-ASPANG, F., MAZZUCO, K. & CHRISTIAN, J. (1969) Der Unterschied in der Induzierbarkeit von Carcinomen zwischen Rücken- und Schwanzhaut der Mäuse in Beziehung zu ihren Schrumpfungsverhalten. *European Journal of Cancer*, **5**, 215.
- SMITH, J.A. & MARTIN, L. (1973) Do cells cycle? *Proceedings of the National Academy of Science of U.S.A.*, **70**, 1263.
- STRAUCH, L. (1972) The role of collagenases in tumour invasion. In: *Tissue Interactions in Carcinogenesis* (Ed. by D.Tarin), p. 399. Academic Press, London.
- TARIN, D. (1972) Morphological studies on the mechanism of carcinogenesis. In: *Tissue Interaction on Carcinogenesis* (Ed. by D.Tarin), p. 227. Academic Press, London.
- VAUGHAN, J.M. (1970) *The Physiology of Bone*. Clarendon Press, Oxford.
- WINTER, G.D. (1964) Regeneration of epidermis. In: *Progress in the Biological Sciences in Relation to Dermatology* (Ed. by A.Rook and R.H.Champion), Vol. II, p. 135. Cambridge University Press.
- WINTER, G.D. (1966) A study of wound healing in the domestic pig, p. 89. Ph.D. Thesis, London University.
- WINTER, G.D. (1972) Epidermal regeneration in the domestic pig. In: *Epidermal Wound Healing* (Ed. by H.I.Maibach and D.T.Rovee), p. 71. Year Book Medical Publishers, Chicago.

Effects of griseofulvin on the morphology, growth and metabolism of fibroblasts in culture

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SUMMARY

Griseofulvin was tested for its effects on the morphology, proliferation and metabolism of fibroblasts cultured from normal human skin and on established lines of mouse fibroblasts 3T3 and 3T6. In all cells treatment produced slight increases in the mitotic index compatible with transient mitotic arrest, but cultures of 3T3 and 3T6 cells also included high proportions (up to 25%) of abnormal multinucleate cells not present in human strains. The proliferation of all cell strains over a 2 or 4 day period was inhibited in proportion to dose (2–17 $\mu\text{g/ml}$) with 50% inhibition of growth in the range of 5–11 $\mu\text{g/ml}$. Acid mucopolysaccharide secretion was depressed by about 20% at 4–17 $\mu\text{g/ml}$. Total protein synthesis was depressed in 3T6 and in human cells, but there was no specific effect on collagen synthesis or secretion.

The antibiotic griseofulvin has shown remarkably low toxicity in its 20 years of dermatological use against fungal infections. Indeed several side effects of potential benefit, including vasodilatation and anti-inflammatory activity, have been described leading to its trial in non-fungal conditions such as Raynaud's syndrome (Allen, 1971), scleroderma (Giordano, 1967), rheumatoid arthritis and shoulder/hand syndrome (Cohen *et al.*, 1960; Serre & Simon, 1962). The mode of action of the drug in such cases is unknown, though some features of structure and activity shared with colchicine have prompted speculation that the inhibition of collagen synthesis might be important. We have therefore tested griseofulvin's effect on connective tissue metabolism using cultured fibroblasts to monitor cell proliferation, collagen synthesis and acid mucopolysaccharide secretion.

METHODS

Cells

3T3 and 3T6 cells were obtained from Gibco-Biocult Limited, Paisley, Scotland, and NCTC 2544 cells from Flow Laboratories, Irvine, Scotland. Human skin fibroblasts, designated HSFI-6 were gifts of Dr A.D. Bain, Royal Hospital for Sick Children, Edinburgh. They had been cultured from normal human foreskin, were predominantly diploid and were used prior to the twentieth passage.

Cell culture

Cells were grown in Falcon plastic flasks unless otherwise indicated. The flasks were seeded with

cells at 1×10^5 , flushed with 5% CO₂ in air and incubated at 37° in Dulbecco's modified Eagle medium with 10% fetal calf serum, 4 mM glutamine, penicillin (100 units/ml) and streptomycin (100 µg/ml) (Gibco-Biocult Limited). The medium was replaced every other day and finally cells were harvested with trypsin-versene for counting in a haemocytometer.

For growth inhibition studies 24 replicate flask cultures were established (Day 0), and on Day 3 four flasks were withdrawn at random and counted as controls. The remaining 20 flasks were organized into 5 groups allowing treatment with 4 concentrations of griseofulvin (2, 4, 8 and 17 µg/ml) and a final control group. Medium containing the drug and 0.1% dimethyl sulphoxide as vehicle was then added, the controls receiving vehicle only. On Day 5 two cultures in each group were counted, while the remainder received fresh control or experimental medium; on Day 7 the remaining cultures were counted. Results were expressed as a percentage of growth in controls over the 2-day (Day 3–5) or 4-day (Day 3–7) exposure to the drug. Because of the low numbers of cultures, the 2 day and 4 day inhibition plots (increase in cell number vs log dose) were pooled into a single plot with each point representing 4 individual values. The concentrations of griseofulvin producing 50% inhibition of cell proliferation were read from plots and designated ID₅₀.

Morphological studies

For morphological studies cells were grown in Rose chambers. After plating at 5×10^4 , cells were treated with griseofulvin on Day 3. Living cells were examined under phase contrast microscopy, after which the cultures were fixed and stained in May-Grunwald and Giemsa. Stained preparations were counted by traversing the whole culture several times, counting all cells which passed through a small circle engraved on the eyepiece. For each of 1000 cells the number of nuclei was recorded, and the stage of mitosis. Values for 3 treated and 3 untreated cultures were combined into treated and control means.

Acid mucopolysaccharide assay

Flask cultures of HSF1 or HSF5 fibroblasts were treated with griseofulvin (2–17 µg/ml) when fully confluent on Day 8. Medium was collected on Day 10 and the cells were harvested and counted. The medium was centrifuged at 1000 rev/min to remove loose cells and debris and then dialysed for 3 days against 0.1 M sodium acetate buffer (pH 4.8) containing 0.1 M sodium chloride. The volume was then reduced to about 4 ml by adding Lyphogel (Gelman-Hawksley, Lancing, Sussex) which absorbs water and small molecules. Each sample was next treated with 2 mg hyaluronidase (Sigma, Type I) overnight at 37°C. The samples were then cooled to 4°C, made up to 5% with trichloroacetic acid (TCA) to precipitate protein and centrifuged at 18,000 g for 30 min. Uronic acid was assayed in supernatants. The modified carbazole method (Bitter & Muir, 1962) proved too unreliable for this material, and the methoxydiphenol method of Blumenkrantz & Asboe-Hansen (1973) was used with a glucuronolactone standard. Unused medium, processed along with experimental samples in every assay, contained 1–1.5 µg uronic acid per ml; this amount was subtracted to give uronic acid output per culture, and, by calculation from cell counts, output per 10⁷ cells.

Collagen synthesis

Collagen and other protein synthesis was assessed in confluent 3T3, 3T6 and HSF6 cultures grown in plastic multi-well dishes (six 3 cm cultures per dish) in medium containing 50 µg/ml ascorbic acid. After 45 min preincubation with the drug in medium without serum or antibiotics but containing ascorbic acid, 10 µCi of L[5-³H]proline (Radiochemical Centre, Amersham) was added to each culture and incubated for 5 h at 37°C. Each dish contained three treated and three control cultures, which were later pooled into one treated and one control preparation. After incubation cultures were

kept at 4°C. Medium was withdrawn and to it was added 1 ml of phosphate buffered saline used to rinse the cells which were then removed with a jet of cold distilled water and homogenized. Carrier protein (1 drop of 1% serum albumin) was added to media samples, and then both cells and media were made up to 5% with TCA at 4°C. Precipitated protein was collected by centrifugation at 2000 rev/min and washed successively with ethanol containing 2% sodium acetate, ethanol-ether (3:1), and ether. The dry pellets were dissolved in 0.5 ml 0.1 N KOH, neutralized with 0.4 N HCl, and diluted with 2 ml Tris-HCl buffer pH 7.6, containing 10 mM calcium chloride and divided into two aliquots. One aliquot was incubated with 100 units of collagenase overnight at 37°C, while the other was incubated with buffer. Protein was next reprecipitated in 5% TCA containing 0.25% tannic acid, washed as before and dried. Residues were solubilized in hyamine hydroxide, acidified with 0.1 ml glacial acetic acid, and added to 10 ml NE 233 scintillation fluid (Nuclear Enterprises, Edinburgh) for liquid scintillation counting at approximately 40% efficiency. A quench correction by external standard channels ratio was used to convert cpm to dpm.

Two different collagenases were used. For 3T3 and 3T6 cells the enzyme was obtained from Sigma (London) Chemical Company (Collagenase Type III). Although 0.5% *n*-ethyl maleimide was always included in the incubation buffer as protease-inhibitor, tests with ³H-tryptophan-labelled chick embryo protein (Peterkofsky & Diegelmann, 1971) still indicated traces of protease activity in this nominally protease-free enzyme. As a further precaution against over-estimation of nascent collagen, the first 10% of the total radioactivity dispersed by the enzyme was disregarded, as non-collagenous, the remainder was regarded as collagenous protein. In later experiments with HSF cells collagenase was obtained from Advance Biofactures, Lynbrook, New York. Since enzyme from this source is without effect on ³H-tryptophan-labelled chick protein (Miller & Udenfriend, 1970) and is therefore free from protease activity, all radioactivity dispersed is in collagenous protein. Relative collagen synthesis (collagen as percentage of total synthesis) was calculated by the formula of Diegelmann & Peterkofsky (1972). Percentage secretion represents the proportion of protein-bound radioactivity present in the medium as opposed to the cell layer.

RESULTS

Griseofulvin depressed the proliferation of all cell lines in a clearly dose-dependent manner. Mean ID₅₀ values for the fibroblast lines were between 5 and 11 µg/ml, with no distinction between the mouse lines 3T3 and 3T6 and the human HSF strains (Fig. 1). The skin epithelial line NCTC 2544 proved more susceptible to griseofulvin at all concentrations, with an ID₅₀ of 3.3 µg/ml. In all cells there was increased inhibition with longer exposure to griseofulvin—4 day treatment giving more inhibition than 2 day.

Morphological observations showed a distinct difference in griseofulvin's effect on cell division in the mouse and human cells. In 3T3 and 3T6 overnight treatment produced large numbers of multinucleate cells, with over 1 in 4 cells affected at 17 µg/ml in 3T3 (Figs 2-4). Affected cells had from 2 to 7 nuclei, often of different sizes. Abnormal elongated cells in mitosis were present and although anaphase and telophase figures were rare or absent, the overall mitotic index was only slightly elevated (Fig. 4) and never exceeded 6%, showing that there was no general or permanent metaphase arrest. The number of multinucleate cells was proportional to dose, but even 2 µg/ml had an effect (Fig. 5). 3T3 and 3T6 cells gave very similar results. However, in the human strains treatment gave no significant increase in multinucleate cells; here again absence of late-stage mitoses suggested some metaphase arrest, but mitotic indices were only slightly raised. In NCTC 2544 cells griseofulvin at 8 µg/ml doubled the number of multinucleate cells (but only from 1.8 to 3.6%) and produced the clearest example of metaphase arrest, with a mitotic index of 16%, against 2% in controls.

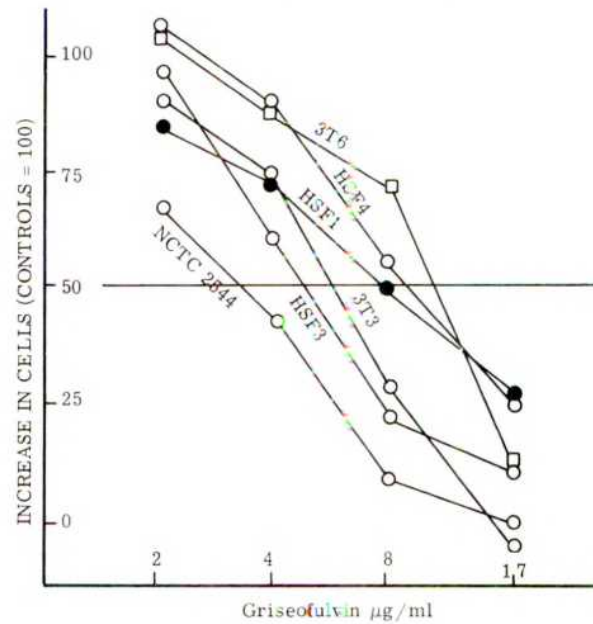


FIGURE 1. Effect of griseofulvin on proliferation of human skin fibroblasts (HSF 1-4), 3T3 and 3T6 mouse fibroblasts and NCTC 2544 skin epithelial cells. The intersection of each plot with the 50% proliferation level was read as the ID_{50} value.

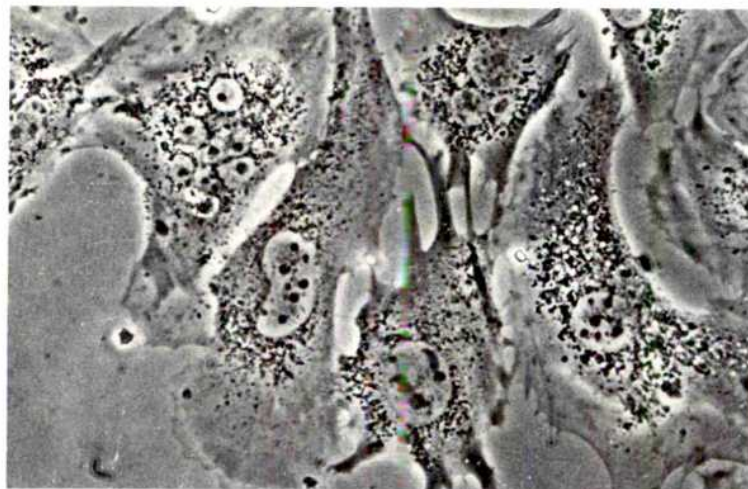


FIGURE 2. A culture of 3T3 fibroblasts treated with 17 µg/ml griseofulvin overnight. Cells with seven and three nuclei are seen among normal mononucleate cells (Phase contrast photograph of living culture, $\times 1250$).



FIGURE 3. A control culture of mononucleate 3T3 cells (Phase contrast, $\times 1250$).

Acid mucopolysaccharide secretion

In Fig. 6 the data for HSF 1 cells show a reduction in acid mucopolysaccharide secretion of just over 20% at from 4 to 17 $\mu\text{g/ml}$, which was statistically significant. The data from HSF 5 cells show essentially the same pattern with a reduction of just under 20% at 8 and 17 $\mu\text{g/ml}$, again statistically significant. Taken together the two curves suggest that secretion is depressed in linear fashion to reach a plateau of about 20% depression at 6–17 $\mu\text{g/ml}$. In absolute terms this represents a reduction from 91 to 76 μg hexuronic acid secreted by 10^7 HSF 1 cells in 48 h and a reduction from 48 to 40 μg for HSF 5

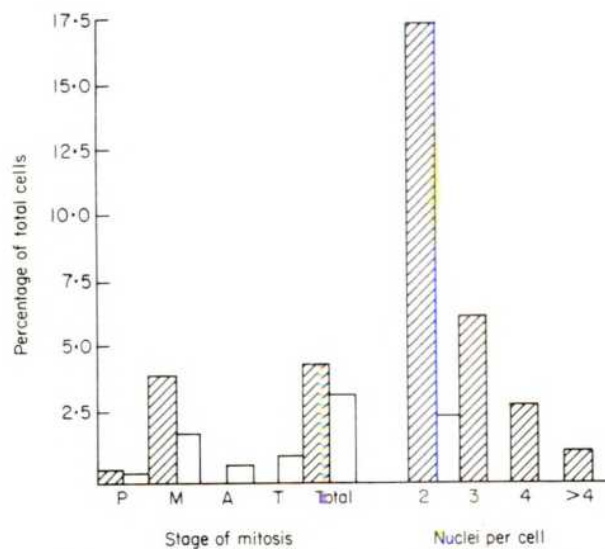


FIGURE 4. Analysis of effects of griseofulvin on 3T3 fibroblasts after overnight treatment at 17 $\mu\text{g/ml}$. Mean values are shown from 3 treated (hatched columns) and 3 control cultures (open columns); each represents a percentage of the total cells. Stages of mitosis are P, prophase; M, metaphase; A, anaphase; T, telophase.

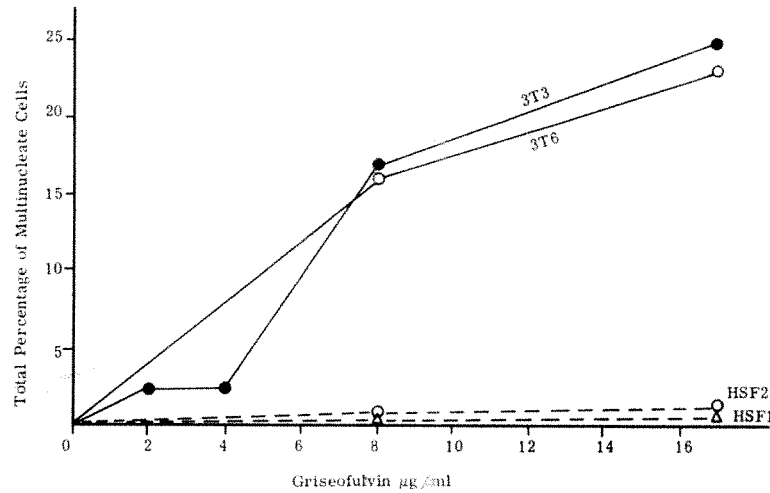


FIGURE 5. Comparison of griseofulvin's effect on total incidence of multinucleate cells in 3T3, 3T6 and human skin fibroblasts HSF 1 and HSF 2. Control values have been subtracted from each total.

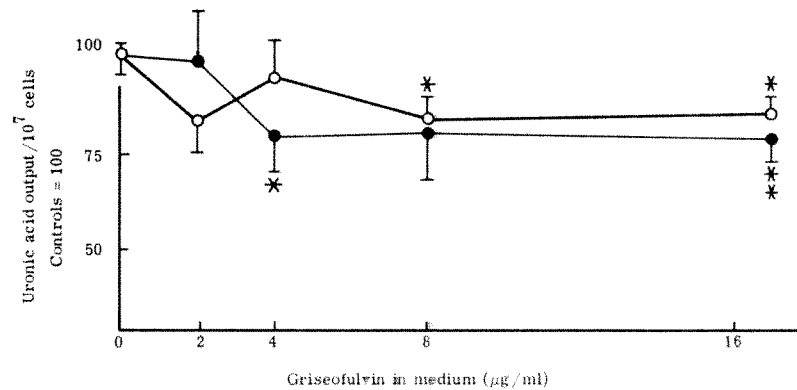


FIGURE 6. Effect of griseofulvin on acid mucopolysaccharide secretion by HSF 1 (closed circles) and HSF 5 (open circles) human skin fibroblasts. Bars indicate one standard error around means; $n = 4$ for HSF 1; $n = 6$ for HSF 5. Asterisks denote $P < 0.05$; double asterisk $P < 0.01$.

cells. This is additional to any change produced by griseofulvin's effect on the number of cells, a factor eliminated in calculating the results in this form.

Collagen

Table 1 shows that griseofulvin had little direct effect upon collagen synthesis: the percentage of labelled collagenous protein was slightly lower in 3T3 and 3T6 cells after treatment, but the results were too variable to be significant. There was no significant effect on collagen secretion.

Protein synthesis generally was depressed by 29% in 3T6 cells, but there was no effect in 3T3. In HSF6 cells incorporation was depressed by 9% at 17 µg/ml and by 14% at 34 µg/ml. All these depressions were statistically significant.

TABLE 1. Effect of griseofulvin on total protein and collagen synthesis

Cells	Treatment	n	Total protein synthesis (dpm)	Collagen as % of total*	% Collagen secreted	% Non-collagen protein secreted
3T6	Control	4	1537131	6.5	5	2
	Gris. 17 µg/ml	4	1098123†	4.9	9	2
3T3	Control	4	217816	5.1	19	8
	Gris. 17 µg/ml	4	235510	4.8	27	6
HSF6	Control	7	97293	4.2	60	17
	Gris. 17 µg/ml	8	89717†	5.0	51	19
	Gris. 34 µg/ml	8	83871‡	4.7	51	15

Each value shown is a mean from n preparations, each preparation from 3 pooled cultures.

* Relative collagen synthesis as calculated by Diegelmann & Peterkofsky (1972).

† $P < 0.05$.

‡ $P < 0.01$ using 't'-test.

DISCUSSION

Our data show several actions of griseofulvin on skin fibroblasts *in vitro*. Griseofulvin can influence the number of fibroblasts, and therefore, indirectly, their output of specific products like collagen, hyaluronic acid or dermatan sulphate. In addition the drug can depress acid mucopolysaccharide synthesis directly, and the degree of depression—about 20%—is equivalent to that produced in the same cells by hydrocortisone, a more potent anti-inflammatory drug (Priestley, 1978). Griseofulvin does not appear to affect collagen synthesis directly, although a modest indirect effect may follow the depression of all fibroblast protein synthesis. This distinguishes griseofulvin from colchicine, a drug with some similarities, but one which blocks collagen secretion in 3T3 cells without affecting other protein synthesis (Diegelmann & Peterkofsky, 1972). The inhibitory effects described here give no cause for alarm in the clinical use of griseofulvin, and in fact may help to explain the beneficial results of griseofulvin therapy claimed in those connective tissue diseases where fibroblast metabolism and output of macromolecules is abnormally high.

Effects recorded *in vitro* do not necessarily occur *in vivo*, and even where they do the matching of drug concentrations in the two situations is largely a matter of guesswork. Peak serum values after griseofulvin ingestion range from 0.9 to 3.3 µg/ml (Platt, 1970), or up to 5 µg/ml when microcrystalline griseofulvin is given with a fat diet (Crounse, 1963; Kraml *et al.*, 1963) but the corresponding concentrations in the dermis are not known; concentrations in whole skin exceed serum values shortly after dosage (Bedford *et al.*, 1960) but a disproportionate amount may be in the epidermis, the stratum corneum or in hair follicles. However, assuming that serum and dermal values are equivalent, then concentrations of the drug with some activity *in vitro*, i.e. 2–4 µg/ml, would come into the clinical range.

Paget & Walpole (1958) showed that in rats high parenteral doses of griseofulvin caused widespread arrest of mitosis in metaphase at sites of high cell turnover, such as mucosa and liver. Recently Weber *et al.* (1976), treating 3T3 cells *in vitro*, reported that in a restricted dose range metaphases accumulated to produce a mitotic index of 50%. Evidence of mitotic arrest in our experiments was limited to a relative scarcity of anaphase and telophase figures, with a marginally higher but still low mitotic index in human fibroblasts. In the skin epithelial cells an effect was clearer, with 16% of the cells in mitosis. In the mouse fibroblasts the main effect was the appearance of numerous multinucleate cells; repeated trials at different drug concentrations failed to produce results resembling those of Weber *et al.* Multinucleate cells have been reported in griseofulvin-treated fungal cells (Gull & Trinci, 1973),

and Paget & Walpole's original report mentions multipolar mitoses 'leading to nuclei of different sizes within the (liver) cell'. The different effect on the HSF strains and 3T3 and 3T6 is probably due to the heteroploid nature of the established lines, rather than the species of origin. In other instances heteroploid and diploid cell lines have reacted differently to the same drug; growth of mouse L cells is inhibited by hydrocortisone and other corticosteroids *in vitro* (Berliner & Ruhmann, 1967), but proliferation of human skin fibroblasts is enhanced by hydrocortisone (Castor, 1962; Kirk & Mittwoch, 1977; Priestley, 1978). In view of these differences in behaviour, and since diploid fibroblasts readily obtained from human skin preserve their differentiated functions of collagen and acid mucopolysaccharide synthesis *in vitro*, the use of established lines as models for normal human fibroblasts no longer seems justified.

ACKNOWLEDGMENTS

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REFERENCES

- ALLEN, B.R. (1971) Griseofulvin in Raynaud's phenomenon. *Lancet*, **ii**, 840.
- BEDFORD, C., BUSFIELD, D., CHILD, K.J., MACGREGOR, I., SUTHERLAND, D. & TOMICH, E.G. (1960) Studies on the biological disposition of griseofulvin, an oral antifungal agent. *Archives of Dermatology*, **81**, 735.
- BERLINER, D.L. & RUHMANN, A.G. (1967) Influence of steroids on fibroblasts. 1. An *in vitro* assay for corticosteroids. *Journal of Investigative Dermatology*, **49**, 117.
- BITTER, T. & MUIR, H. (1962) A modified uronic acid carbazole reaction. *Analytical Biochemistry*, **4**, 330.
- BLUMENKRANTZ, N. & ASBOE-HANSEN, G. (1973) New method for quantitative determination of uronic acids. *Analytical Biochemistry*, **54**, 484.
- CASTOR, C.W. (1962) Adrenocorticoid suppression of polysaccharide formation in human connective tissue cell cultures. *Journal of Laboratory and Clinical Medicine*, **60**, 788.
- COHEN, A., GOLDMAN, J., DANIELS, R. & KANENSON, W. (1960) Treatment of shoulder-hand syndrome with griseofulvin. *Journal of the American Medical Association*, **173**, 542.
- CROUNSE, R.G. (1963) Effective use of griseofulvin. *Archives of Dermatology*, **87**, 176.
- DIEGELMANN, R.F. & PETERKOFKY, B. (1972) Inhibition of collagen secretion from bone and cultured fibroblasts by microtubular disruptive drugs. *Proceedings of the National Academy of Sciences, U.S.A.*, **69**, 892.
- GIORDANO, M. (1967) Griseofulvin for scleroderma. *Lancet*, **ii**, 260.
- GULL, R. & TRINCI, A.P.J. (1973) Griseofulvin inhibits fungal mitosis. *Nature*, **244**, 292.
- KIRK, D. & MITTVOCH, U. (1977) Effects of topically potent glucocorticoids on human diploid fibroblasts *in vitro*. *British Journal of Dermatology*, **97**, 69.
- KRAML, M., DUBUC, J. & DVORNIK, D. (1963) Gastrointestinal absorption of griseofulvin. *Archives of Dermatology*, **87**, 179.
- MILLER, R.L. & UDENFRIEND, S. (1970) Hydroxylation of proline residues in collagen nascent chains. *Archives of Biochemistry and Biophysics*, **139**, 104.
- PAGET, G.E. & WALPOLE, A.L. (1958) Some cytological effects of griseofulvin. *Nature*, **182**, 1320.
- PETERKOFKY, B. & DIEGELMANN, R. (1971) Use of a mixture of proteinase-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. *Biochemistry*, **10**, 988.
- PLATT, D.S. (1970) Plasma concentrations of griseofulvin in human volunteers. *British Journal of Dermatology*, **83**, 382.
- PRIESTLEY, G.C. (1978) Effects of corticosteroids on the growth and metabolism of fibroblasts cultured from human skin. *British Journal of Dermatology*, **99**, 253.
- SERRE, H. & SIMON, L. (1962) Unexpected therapeutic action of an antibiotic fungicide (griseofulvin) in rheumatology. *Presse Medicale*, **70**, 2263.
- WEBER, K., WEHLAND, J. & HERZOG, W. (1976) Griseofulvin interacts with microtubules both *in vivo* and *in vitro*. *Journal of Molecular Biology*, **102**, 817.

Effects of corticosteroids on the growth and metabolism of fibroblasts cultured from human skin

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SUMMARY

Hydrocortisone, betamethasone 17-valerate, clobetasone butyrate and clobetasol propionate were tested *in vitro* for effects on the proliferation and metabolism of six strains of fibroblasts from normal human skin.

The different cell strains gave similar results: hydrocortisone and betamethasone valerate slightly enhanced growth at all concentrations (0.0001–10 µg/ml), but high concentrations of clobetasone butyrate and clobetasol propionate significantly reduced proliferation.

Secretion of acid mucopolysaccharide was most inhibited by clobetasone butyrate and clobetasol propionate, then by betamethasone valerate, with hydrocortisone having the least effect. Clobetasol propionate and betamethasone valerate at 10 µg/ml both reduced collagen synthesis by about 50%; other protein synthesis was less affected.

Cell culture permits the investigator to make quantitative measurements of several metabolic activities within a single cell type and any changes which occur when the cells are exposed to drugs can be recorded. Skin epithelial cells or skin fibroblasts, for instance, can be treated with drugs at precise concentrations. The technique can therefore provide more detailed and more objective study of drug effects than is possible from clinical assessment or histological study of whole skin. In this paper we compare the effects of four corticosteroids, all in current clinical use, on fibroblasts prepared from normal human skin.

METHODS

Materials

Cell strains derived from normal human foreskin were obtained from the Royal Hospital for Sick Children, Edinburgh, and designated strains HSF 1–6. They were used for assessment prior to the twentieth passage. Steroids were obtained in identical vials labelled A–D and were used without knowledge of their identity for tests on HSF 1 and HSF 3 strains, after which the code was broken. The compounds were dissolved in 1:1 dimethyl sulphoxide-propylene glycol for addition to culture media, control cultures receiving this vehicle only at a maximum combined concentration of 0.1%.

Cell culture

Cells were routinely cultured in Falcon plastic flasks. Freshly seeded cell suspensions (1×10^5 cells per 25 cm² flask) were flushed with 5% carbon dioxide in air and incubated at 37°C in Dulbecco's version of Eagle's medium (Gibco-Biocult Ltd., Paisley, Scotland) supplemented with 4 mM glutamine, penicillin (100 units per ml), streptomycin (100 µg/ml) and 10% fetal calf serum. The medium was changed on alternate days and cells were finally harvested with trypsin-versene for counting in a haemocytometer.

Cell proliferation

Thirty-two flask cultures were established (Day 0), and on Day 3 four flasks were withdrawn at random and the cells counted as controls. The remaining flasks were divided into seven groups providing for treatment with six different concentrations of a single steroid plus a further control group, and medium containing the appropriate drug concentrations was then added. On Day 5 the cells in two flasks in each group were counted, while the rest received fresh medium; on Day 7 all the remaining cultures were harvested and counted. Results were expressed as a percentage of growth (increase in number of cells) in the controls over the 2-day (Days 3-5) or 4-day (Days 3-7) exposure to the drug. Because of the small numbers of cultures, 2 day and 4 day data were combined into a single plot of cell proliferation vs log dose with four values for each concentration of drug.

Multi-steroid experiments, with all four compounds at a single concentration (10 µg/ml), were organized along the same lines, but starting with only 24 flasks, and employed a single exposure time of 4 days (Days 3-7).

Acid mucopolysaccharide assay

Medium from flask cultures in proliferation experiments, added on Day 5 and collected on Day 7, was analysed by the procedure previously described (Priestley & Brown, 1978). Briefly, the medium was dialysed, concentrated, digested with hyaluronidase, acidified to precipitate protein, and uronic acid was then estimated in the supernatant using metahydroxydiphenol (Blumenkrantz & Asboe-1973). Uronic acid present in unused medium was measured and deducted in calculating output per culture, and output per 10^7 cells was calculated from cell counts.

Protein synthesis

Total protein and collagen synthesis were estimated, as in the previous paper (Priestley & Brown, 1978), by an adaptation of the method of Diegelmann & Peterkofsky (1972). In principle, total protein synthesis is recorded as incorporation of ³H-labelled proline into acid-precipitable material, and non-collagen protein synthesis as the radioactivity remaining after digestion of the labelled total protein with protease-free collagenase. The amount of labelled collagen is arrived at by difference.

Cultures of HSF 4 and HSF 6 strains (both mycoplasma-free) were allowed to incorporate L[5-³H] proline for 5 h after a 45 min preincubation with steroid or vehicle, as previously described (Priestley & Brown, 1978), and the labelled protein in the homogenized cells and medium was prepared, washed and dried as before. The precipitates were redissolved, added to 2 ml of Tris-HCl buffer (pH 7.4) containing 10 mM calcium chloride and divided into two equal aliquots. To one of these, 100 units of collagenase (Advance Biofactures Corporation, Lynbrook, U.S.A.) was added, and all samples were incubated at 37°C overnight. Protein was then reprecipitated and prepared for scintillation counting as described by Priestley and Brown (1978).

Since collagenase from this source is protease-free (Miller & Udenfriend, 1970), all protein-bound radioactivity dispersed by the enzyme was regarded as collagen. 'Relative collagen synthesis'—

the percentage of the total protein synthesis which is collagen—was calculated by the method of Diegelmann & Peterkofsky (1972).

RESULTS

Cell proliferation

The steroids rarely gave the straight line plots of cell increase against the logarithm of the dose which we have obtained with non-steroid drugs in the same cell strains (Priestley & Brown, 1978) and which others have reported in tests with steroids on established lines of non-human cells (Berliner & Rhu-
mann, 1965; Brotherton, 1971). Dose response experiments with HSF 1 and HSF 3 cells showed two contrasting patterns of response: clobetasone butyrate and clobetasol propionate depressed proliferation at high dose levels but betamethasone valerate and hydrocortisone actually enhanced proliferation

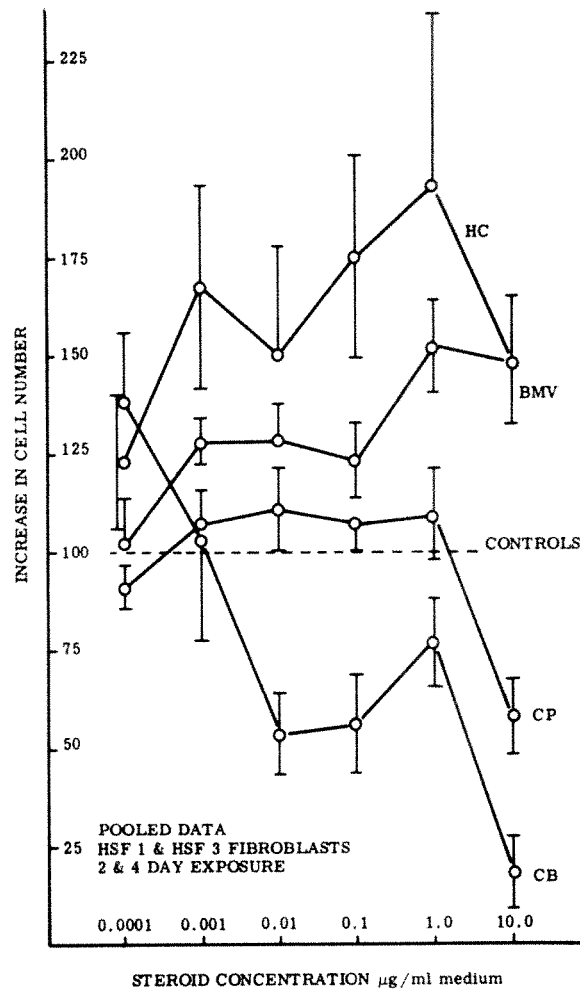


FIGURE 1. Effect of steroids on HSF 1 and HSF 3 skin fibroblast proliferation, performed without knowledge of each steroid's identity. Each point represents 8 individual values, four from each cell strain; standard errors are indicated. Growth of controls is shown as 100. The steroids were: HC, hydrocortisone; BMV, betamethasone valerate; CP, clobetasol propionate; CB, clobetasone butyrate.

TABLE 1. Effect of steroids at highest dosage (10 µg/ml) on proliferation of human skin fibroblasts. Results expressed as a percentage of increase in control cells. Mean values from 4 cultures

Cells	Type of experiment	Steroid			
		CB	BV	HC	CP
HSF 1	Dose-response*	43	139	138	75
HSF 1	Multisteroid†	45	110	102	46
HSF 3	Dose-response*	-6	156	158	42
HSF 3	Multisteroid†	42	529	373	67
HSF 2	Multisteroid†	60	116	109	36
HSF 5	Multisteroid†	67	101	125	52
HSF 6	Multisteroid†	38	89	111	33
Pooled means (n = 24) ± s.e.m. (Excluding HSF 3 MS)		41 ± 7	118 ± 8	124 ± 7	47 ± 4

* One steroid tested at several concentrations.

† All 4 steroids tested at 10 µg/ml only.

(Fig. 1). The effects were roughly related to dose except with clobetasol propionate. Further tests, with all four steroids at 10 µg/ml only, on the same cell strains confirmed this pattern, with good quantitative agreement on the effect of each compound at 10 µg/ml, except where the growth of HSF 3, a slow-growing strain, was enhanced disproportionately (Table 1).

Tests of all four compounds at 10 µg/ml on three more cell strains continued the same pattern of results. There were slight variations in the ranking of each compound within its pair, but differences between the two pairs were always large and, in the final pooled data from all cell strains (Table 1), highly significant statistically ($P < 0.001$ in 't'-test).

Acid mucopolysaccharide secretion

In dose-response experiments with HSF 1 cells, all four compounds inhibited secretion of acid mucopolysaccharide (Fig. 2). The clearest dose-relation was with clobetasol propionate ($r = 0.89$; $P < 0.001$ at 10 degrees of freedom), and clobetasone butyrate ($r = 0.7$; $P < 0.02$ at 10 df).

To compare the effects of the steroids more effectively the results obtained at 10 µg/ml were pooled with those from a multisteroid experiment (also at 10 µg/ml) to give six values for each steroid, as shown in Fig. 3. Secretion per culture was most reduced by clobetasol propionate and clobetasol butyrate, then by betamethasone valerate, and least by hydrocortisone. HSF 5 cells treated at 10 µg/ml gave the same pattern of results (not shown), although the overall reduction in secretion was slightly greater.

Because differences in mucopolysaccharide secretion might reflect differences merely in the number of cells in cultures treated with steroids for two days (Fig. 1 and Table 1), the results in Fig. 3 are also expressed as secretion per 10^7 cells, using the final cell numbers counted when the medium was collected to calculate the adjusted values. However, this did not alter the pattern of the results for either cell strain, or the ranking of the four compounds (Fig. 3 and Table 2).

Protein synthesis

Table 3 shows that treatment with steroids at 10 µg/ml had little or no effect on general protein synthesis, yet clobetasol propionate decreased incorporation into collagen by 42%.

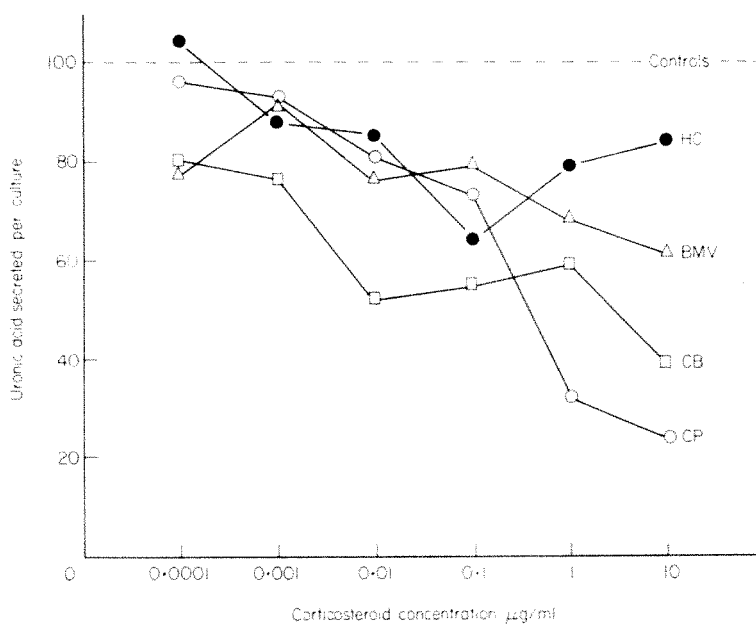


FIGURE 2. Inhibition of acid mucopolysaccharide secretion by various concentrations of steroid. Each value is a mean from 2 cultures, expressed as a percentage of output in untreated controls.

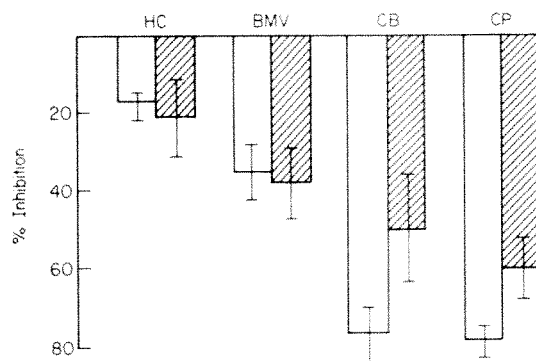


FIGURE 3. Inhibition of acid mucopolysaccharide secretion in HSF 1 cells by 10 µg/ml steroid. Values are means with standard errors ($n = 6$); all except that for output per 10^7 cells with HC were significantly different from controls ($P < 0.05$). Open columns, output/flask; hatched columns, output/ 10^7 cells.

To try to increase the effects the steroid concentration was raised to 25 µg/ml. The alternative, to lengthen the labelling time from 5 h, would have involved longer treatment with the steroids and increasing risk of altering the number of cells in each culture through effects on proliferation. At 25 µg/ml both betamethasone valerate and clobetasol propionate inhibited collagen synthesis by more than 50%, with smaller but still notable inhibition of all protein synthesis and suggestive decreases in collagen as a percentage of the total.

These results show that both clobetasol propionate and betamethasone valerate can inhibit the synthesis of collagen and other proteins by fibroblasts. The finding that collagen synthesis was depressed appreciably more than synthesis of other proteins argues for some specificity of the effect.

TABLE 2. Effect of steroids at 10 µg/ml on acid mucopolysaccharide (AMPS) secretion. Analysis of pooled results from HSF 1 and HSF 5 cells (n = 10)

Group	% inhibition of AMPS output/10 ⁷ cells ± s.e.	't'-test comparison with:		
		Controls	HC	BMV
Controls	0 ± 6			
HC	27 ± 8	<i>P</i> < 0.01		
BMV	45 ± 8	< 0.01	> 0.1	
CB	62 ± 10	< 0.01	< 0.01	> 0.1
CP	65 ± 7	< 0.01	< 0.01	< 0.05

TABLE 3. Effect of corticosteroids on protein synthesis

Treatment	Collagen synthesis (%)	All protein synthesis (%)	Collagen synthesis as % of total
(A) At 10 µg/ml; HSF 4 cells (n = 7)			
Controls	100 ± 8	100 ± 3	4.1 ± 0.5
CB	92 ± 11	94 ± 5	4.3 ± 0.7
BMV	108 ± 23	103 ± 3	3.9 ± 0.6
HC	84 ± 21	106 ± 6	3.0 ± 0.8
CP	58 ± 12†	94 ± 6	2.5 ± 0.6
(B) At 25 µg/ml; HSF 6 cells (n = 7)			
Controls	100 ± 15	100 ± 5	3.3 ± 0.5
CB	100 ± 9	94 ± 5	3.6 ± 0.3
BMV	48 ± 14*	73 ± 6‡	2.1 ± 0.5
HC	89 ± 13	97 ± 6	3.0 ± 0.4
CP	47 ± 13*	63 ± 5‡	2.4 ± 0.6

Mean values are shown with standard errors.

Comparisons by 't'-test: * *P* < 0.05; † *P* < 0.02; ‡ *P* < 0.01.

DISCUSSION

The three inhibitory actions of corticosteroids reported here—on cell proliferation, acid mucopolysaccharide secretion, and collagen synthesis—are characteristic of many anti-inflammatory and anti-rheumatic drugs. In the present study four compounds in current dermatological use were tested for all three inhibitory functions using several strains of normal human skin fibroblasts. Table 4 summarises the different abilities of each drug. In more detail, the results confirm Castor's (1962) finding that hydrocortisone may stimulate human fibroblast growth while simultaneously depressing acid mucopolysaccharide secretion; betamethasone valerate appears to have the same ability. Several authors have studied the effects of steroids on collagen synthesis, with rather similar results to those shown here. Uitto, Teir & Mustakallio (1972) found that betamethasone valerate gave the most pronounced

TABLE 4. Summary of inhibitory effects

Inhibition	CP	CB	BMV	HC
Cell proliferation	++	++		
AMPS secretion	++	++	+	+
Collagen synthesis	++		++	

inhibition *in vitro* but all the compounds tested were inhibitory at concentrations of 30 µg/ml or more. Saarni & Hopsu-Havu (1976) found that betamethasone valerate depressed collagen synthesis in chick tendon by 90% at 10^{-4} M (about 36 µg/ml); prednisolone had a 20–30% effect and hydrocortisone succinate virtually none. Slight stimulation of collagen synthesis was seen with lower hydrocortisone levels (Saarni & Hopsu-Havu, 1976; Harvey, Grahame & Panayi, 1974). Uitto & Mustakallio (1971) concluded that collagen synthesis is inhibited by a non-specific inhibition of polypeptide assembly, other proteins being equally affected. Our results suggest that clobetasol propionate and betamethasone 17-valerate may affect collagen synthesis more than synthesis of other proteins (Table 3), and this suggestion is now supported by Newman & Cutroneo's more conclusive evidence of triamcinolone diacetate's selective action against collagen synthesis in rats (Newman & Cutroneo, 1978). Clinical experience shows that both clobetasol propionate and betamethasone valerate can produce serious thinning of the skin when used at high dosage.

These data are a guide to the capacity of each steroid, in pure form, to affect specific aspects of dermal metabolism. Because the *in vitro* test system is artificial the data cannot be used to predict the optimum levels (with maximum anti-inflammatory effect and minimum side effects) of each drug in the skin. Although concentrations used *in vitro* are not necessarily equivalent in potency to the same concentrations *in vivo* (Uitto & Mustakallio, 1971; Ebert & Prockop, 1967) those used in our experiments relate to clinical use. Franz and his colleagues (1977) found the steroid concentration in the dermis 90 min after topical application of 1% hydrocortisone to normal skin to be 6 µM (equivalent to 2.2 µg/ml). Since this was increased almost ninety-fold with the stratum corneum removed, the greater penetration through diseased skin must produce still higher concentrations and our highest levels of 10 and 25 µg/ml are not unrealistic.

Dermal atrophy is a serious disadvantage of treatment with potent corticosteroids and the inhibitory effects described here may contribute to this. Control of fibroblast numbers itself implies an indirect control of all fibroblast products, including collagen, elastin, reticulin and acid mucopolysaccharides. Since collagen represents so much of the dermis, while mucopolysaccharides such as hyaluronic acid are largely responsible for its water-binding capacity, a reduced production of these macromolecules will inevitably affect skin thickness, although effects on catabolism may be equally (or even more) important (Houck & Sharma, 1968).

It seems unlikely that the effects described here are peculiar to the *in vitro* model. Young, Yoxall & Wagner (1977) found that the dry weight, collagen, acid mucopolysaccharide and water contents of the epidermis were reduced in rats treated topically with triamcinolone acetonide. Comparisons of the steroids used in the present study for their effect on human skin thickness (Kirby & Munro, 1976) showed that clobetasol propionate (as Dermovate) produced the greatest thinning, clobetasone butyrate (as Molivate) and betamethasone valerate (as Betnovate) produced moderate thinning and hydrocortisone (as Dioderm) produced least thinning—a similar ranking to the overall inhibitory ranking found in the present study (Table 4). Evidence that steroids affect fibroblast proliferation *in vivo* might be difficult to obtain because of the sparsity of the cells in the dermis, but there is considerable

evidence that corticosteroids inhibit the proliferation of human epidermis (Marks, Halprin, Fukui & Graff, 1971; Fisher & Maibach, 1971).

These experiments show both qualitative and quantitative differences between the four compounds (Table 4) within the dose-range studied. This contrasts with the prevailing view that corticosteroids differ only in potency but have the same pattern of actions collectively magnified or diminished. The exploitation of such qualitative differences offers hope of more effective corticosteroid therapy in the future, but clearly more detailed data on the effect of each compound are required.

Berliner first suggested that fibroblast inhibition by a drug was related to its overall anti-inflammatory potency, and proposed the method as a screen for new compounds (Berliner & Ruhmann, 1967) as an alternative to the vasoconstriction test (Mackenzie & Stoughton, 1962). Berliner's experiments with L cells—an established heteroploid and potentially cancerous cell line derived from mice—produced mathematically elegant results which have been confirmed by others (Brotherton, 1971). Unfortunately, diploid human fibroblasts give different results (Castor, 1962; Kirk & Mittvovh, 1977) so that despite the convenience of working with established cell lines they are an unreliable model for normal human skin cells.

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REFERENCES

- BERLINER, D.L. & RUHMANN, A.G. (1967) Influence of steroids on fibroblasts. An *in vitro* assay for corticosteroids. *Journal of Investigative Dermatology*, **49**, 117.
- BLUMENKRANTZ, N. & ASBOE-HANSEN, G. (1973) New method for quantitative determination of uronic acids. *Analytical Biochemistry*, **54**, 484.
- BROTHERTON, J. (1971) Parallel-line biological assay of anti-inflammatory steroids using skin fibroblasts. *Cytobios*, **3**, 225.
- CASTOR, C.W. (1962) Adrenocorticoid suppression of polysaccharide formation in human connective tissue cell cultures. *Journal of Laboratory and Clinical Medicine*, **60**, 788.
- DIEGELMANN, R.F. & PETERKOFKY, B. (1972) Inhibition of collagen secretion from bone and cultured fibroblasts by microtubular disruptive drugs. *Proceedings of the National Academy of Sciences, U.S.A.*, **69**, 892.
- EBERT, P.S. & PROCKOP, D.J. (1967) Influence of cortisol on the synthesis of sulfated mucopolysaccharides and collagen in chick embryos. *Biochimica et Biophysica Acta*, **136**, 45.
- FISHER, L.B. & MAIBACH, H.I. (1971) The effect of corticosteroids on human epidermal mitotic activity. *Archives of Dermatology*, **103**, 39.
- HARVEY, W., GRAHAME, R. & PANAYI, G.S. (1974) Effects of steroid hormones on human fibroblasts *in vitro*. I. Glucocorticoid action on cell growth and collagen synthesis. *Annals of Rheumatic Diseases*, **33**, 437.
- HOUCK, J.C. & SHARMA, V.K. (1968) Induction of collagenolytic and proteolytic activities in rat and human fibroblasts by anti-inflammatory drugs. *Science*, **161**, 1361.
- KIRBY, J.D. & MUNRO, D.D. (1976) Steroid-induced atrophy in an animal and human model. *British Journal of Dermatology*, **94**, Supplement **12**, 111.
- KIRK, D. & MITTVOVH, V. (1977) Effects of topically potent glucocorticoids on human diploid fibroblasts *in vitro*. *British Journal of Dermatology*, **97**, 69.
- KRANZ, G., SCHAEFER, H. & ZESCH, A. (1977) Hydrocortisone (Cortisol) concentration and penetration gradient. *Acta dermato-venereologica*, **57**, 269.
- MACKENZIE, A.W. & STOUGHTON, R.B. (1962) Method of comparing percutaneous absorption of steroids. *Archives of Dermatology*, **86**, 608.
- MARKS, R., HALPRIN, K.M., FUKUI, K. & GRAFF, D. (1971) Topically applied triamcinolone and macromolecular synthesis by human epidermis. *Journal of Investigative Dermatology*, **56**, 470.

- MILLER, R.L. & UDENFRIEND, S. (1970) Hydroxylation of proline residues in collagen nascent chains. *Archives of Biochemistry and Biophysics*, **139**, 104.
- NEWMAN, R.A. & CUTRONEO, K.R. (1978) Glucocorticoids selectively decrease the synthesis of hydroxylated collagen peptides. *Molecular Pharmacology*, **14**, 185.
- PRIESTLEY, G.C. & BROWN, J.C. (1978) Effects of griseofulvin on the morphology, growth and metabolism of fibroblasts in culture. *British Journal of Dermatology*, **99**, 245.
- SAARNI, H. & HOPUSU-HAVU, V.K. (1976) Effect of hydrocortisone butyrate on collagen synthesis. *British Journal of Dermatology*, **95**, 567.
- UITTO, J., TEIR, H. & MUSTAKALLIO, K.K. (1972) Corticosteroid-induced inhibition of the biosynthesis of human skin collagen. *Biochemical Pharmacology*, **21**, 2161.
- UITTO, J. & MUSTAKALLIO, K.K. (1971) Effect of hydrocortisone acetate, fluocinolone acetonide, flucilorolone acetonide, betamethasone-17-valerate and fluprednylidene-21-acetate on collagen biosynthesis. *Biochemical Pharmacology*, **20**, 2495.
- YOUNG, J.M., YOXALL, B.E. & WAGNER, B.M. (1977) Corticosteroid-induced dermal atrophy in the rat. *Journal of Investigative Dermatology*, **69**, 458.

Leukocyte migration agarose test (LMAT) in sarcoidosis using Kveim test material

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SUMMARY

The two-stage leukocyte migration agarose test (indirect LMAT), shown to be a sensitive *in vitro* assay for cell-mediated immunity, was used to study Kveim reactivity *in vitro* in patients with sarcoidosis. No Kveim-induced inhibition of leukocyte migration in agarose or Kveim-induced lymphocyte transformation *in vitro* was found in 23 patients with sarcoidosis, suggesting that the Kveim reaction is not an expression of cell-mediated immunity.

The leukocyte migration agarose test (LMAT) described by Clausen (1971) has recently been used to study cell-mediated immunity in humans (Clausen, 1971; Astor *et al.*, 1973; Mygind & Stenbjerg, 1974; Koonakosit & Petchclai, 1975; Hoffman *et al.*, 1975; Frei, Erard & Zinkernagel, 1973; Passaleva, Forti & Ricci, 1974; Tautz, Khuen & Ax, 1974; Erard, 1974; Anders & Natvig, 1976; Wilson *et al.*, 1977; Bergstrand, Kallen & Nilsson, 1974; Fleer *et al.*, 1976) and in guinea-pigs (Hoffman, Spitler & Hsu, 1976). Using the direct technique in which antigen is incubated directly with migrating peripheral blood leukocytes, a significant correlation between the presence of migration inhibition and a delayed cutaneous hypersensitivity reaction has been observed for a number of antigens, including purified protein derivative of tuberculin (PPD) (Clausen, 1971; Astor *et al.*, 1973; Mygind & Stenbjerg, 1974; Koonakosit & Petchclai, 1975; Hoffman *et al.*, 1975; Frei, Erard & Zinkernagel, 1973; Passaleva, Forti & Ricci, 1974; Tautz, Khuen & Ax, 1974; Erard, 1974; Anders & Natvig, 1976; Wilson *et al.*, 1977), streptokinase-streptodornase (Astor *et al.*, 1973; Hoffman *et al.*, 1975), candida

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(Astor *et al.*, 1973; Hoffman *et al.*, 1975), coccidioidin (Astor *et al.*, 1973; Hoffman *et al.*, 1975; Wilson *et al.*, 1977), and mumps virus antigen (Anders & Natvig, 1976). However, contradictory findings using PPD and bacillus Calmette-Guerin (BCG) in the direct LMAT have also been reported (Bergstrand, Kallen & Nilsson, 1974; Fleer *et al.*, 1976).

In the two-stage LMAT technique (indirect LMAT), supernatants from cultures of sensitive peripheral blood lymphocytes stimulated with the specific antigen are tested for migration inhibitory activity on polymorphonuclear leukocytes (or leukocytes) from a different donor. The inhibition is caused by a soluble factor which has been shown to be different from macrophage migration inhibition factor (MIF) in guinea-pigs (Hoffman, Spitler & Hsu, 1976) and in man (Rocklin, 1974, 1975; Lomnitzer, Rabson & Koornhof, 1975; Hoffman *et al.*, 1977). This factor, called leukocyte inhibition factor (LIF) (Hoffman, Spitler & Hsu, 1975; Rocklin, 1974), specifically inhibits the migration of polymorphonuclear leukocytes (Hoffman, Spitler & Hsu, 1975; Rocklin, 1974). Clausen (1973) first reported that the indirect LMAT was more sensitive than the direct LMAT, and that the degree of migration inhibition in the indirect technique correlated not only with the presence of skin reactivity to PPD but also with the size of the skin test reaction. These findings have been confirmed by Anders & Natvig (1976), who used PPD and mumps virus as antigens.

Inhibition of migration induced by Kveim material in the direct LMAT has been studied by Zweiman & Israel (1976), who found inhibition of leukocyte migration more often in patients with sarcoidosis than in controls. The differences were not striking, however, and there was considerable overlap. Hardt *et al.* (1976), using Danish Kveim material, observed no migration inhibition in the direct LMAT in 46 sarcoid patients. The more sensitive indirect LMAT has not been used previously to study Kveim reactivity *in vitro*. No Kveim-induced inhibition of leukocyte migration under agarose or Kveim-induced lymphocyte transformation *in vitro* was found in patients with sarcoidosis in the present study.

MATERIALS AND METHODS

Patients. Of the 26 sarcoid patients studied, 18 were females and 8 males, ages 20–49 years (mean 31.0 years). The diagnosis of sarcoidosis was confirmed in all cases by biopsy. According to the duration of the disease it was classified as acute in 7 patients (<3 months), subacute in 7 patients (3–24 months), or chronic in 12 patients (>24 months). The disease was classified as active in cases in which the lesions were progressive or in which the pulmonary lesions had remained unchanged for less than 1 year, and as inactive when the lesions had not progressed during a period of more than 1 year. Twenty-two patients had active sarcoidosis and 4 were in an inactive stage of the disease. According to the pulmonary changes the disease was classified into stages I (6 patients), II (12 patients), and III (8 patients) (Siltzbach, 1967). Six of the patients were on steroids (15–30 mg of prednisone every second day); in these patients the blood samples were taken two days after the last dose of the steroid. Of 13 patients skin tested with 5 TU or PPD (Parke-Davis), 12 were negative to this dose.

Kveim suspensions. Sarcoid spleen suspension (sarcoid spleen B lot 2A) was used as Kveim material. Spleen suspension was prepared as the standard Kveim preparation of Chase-Siltzbach Type 1, but without heating and without preservative. The same sarcoid spleen suspension for *in vivo* studies had been shown to provide a Kveim suspension which in cutaneous tests yielded the expected proportions of positive reactions among patients at various stages of sarcoidosis and a negligible number among patients with other diseases (Siltzbach, 1976). Control test material consisted of spleen suspensions from a normal subject and from a patient with Hodgkin's disease. These suspensions were prepared in the same way as the sarcoid spleen suspensions.

Lymphocyte cultures. The method is described in detail elsewhere (Horsmanheimo *et al.*, 1977). Peripheral blood buffy coat cells were collected after dextran sedimentation, and mononuclear cells were separated on a Ficoll-Isopaque gradient. The mononuclear cells were washed and the cell concentration was adjusted to 3×10^5 cells/ml. One ml cultures were set up in tissue culture tubes in medium RPMI 1640 with 10% inactivated FCS. Sarcoid spleen suspension was used as Kveim material. Control test material consisted of spleen suspension from a normal subject and from a patient with Hodgkin's disease. Spleen suspensions were tested first in 3 patients with sarcoidosis and in 2 healthy controls at concentrations of 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$. No differences were found with the two concentrations, and in further experiments spleen suspensions were used at 10 $\mu\text{g/ml}$. Control cultures were set up without spleen suspensions. All cultures were set up in triplicate and harvested after 6 days. Four hours before the cultures were harvested 1 μCi of [^3H]dThd (New England Nuclear Corporation, specific activity 6.7 Ci/mmol) was added to the culture. The cultures were harvested with an automatic cell harvester, and [^3H]dThd incorporation into DNA was counted by scintillation spectrometer. The results are expressed as a stimulation index (SI) (mean counts per min incorporated in test cultures divided by that in control cultures).

Leukocyte migration agarose test (LMAT)

Direct LMAT. Buffy coat cells from peripheral blood were collected after dextran sedimentation, washed, and resuspended in medium RPMI 1640 containing 10% FCS and 100 $\mu\text{g/ml}$ of different spleen suspensions. Controls were set up without spleen suspensions. The cell concentration was 2.4×10^8 buffy coat cells per ml and the incubation time was 30 min at 37°C. After the incubation the cell suspensions were tested immediately on agarose plates as described below.

Indirect LMAT. Preparation of lymphocyte culture supernatants. Peripheral mononuclear cells were prepared as described for lymphocyte cultures. Cell concentration in cultures from which the supernatants were tested for LIF activity was 2×10^6 mononuclear cells per ml. Spleen suspensions were used first in three patients with sarcoidosis and in two controls at concentrations of 10 and 100 $\mu\text{g/ml}$. No differences were found in the LMAT results for the two concentrations, and in further experiments the spleen suspensions were used at 100 $\mu\text{g/ml}$. The control cultures were set up without spleen suspensions. Supernatants were harvested on the third day and used as such for incubation with indicator cells as described below. Supernatants from control cultures were used as such and after reconstitution with spleen suspensions, so that the test and control supernatants of each pair contained the same concentration of spleen suspension.

Assay of supernatants. Polymorphonuclear (PMN) leukocytes were used as indicator cells in the agarose assay. PMN leukocytes were isolated from the peripheral blood of healthy blood donors as described above. After sedimentation in a Ficoll-Isopaque gradient, the PMN cells from the bottom of the tube were washed with Dulbecco's PBS. Red blood cells were submitted to hypotonic lysis for 45 s, and the cell pellet was washed twice and resuspended in supernatants as described above. The cell concentration of PMN leukocytes was 1.5×10^8 cells/ml and the incubation time with supernatants was 30 min at 37°C. After incubation the cell suspensions were tested on agarose plates as indicated below.

Preparation of agarose plates. Migration of leukocytes was assayed in 1% agarose (Indubiose A37 Agarose, L'Industrie Biologique Francaise S.A.) in medium TC 199 supplemented with 10% horse serum (Gibco, U.S.A.), and with penicillin and streptomycin (Gibco), 60 U and 60 $\mu\text{g/ml}$

respectively, and buffered with sodium bicarbonate. Six ml of agarose medium was poured into disposable 60 mm plastic Petri dishes (3002 Tissue Culture Dish, Falcon, USA) and allowed to solidify. Eight to nine wells 2.3 mm in diameter were punched with a stainless steel punch, and 7- μ l samples of the various cell suspensions were placed in the wells. Each cell suspension was run in triplicate or in quadruplicate. The plates were placed in inverted 100-mm plastic Petri dishes, which were sealed with sterile water and incubated for 18 h at 37°C in an atmosphere of 5% carbon dioxide and 95% air. Two diameters of migration area were measured with a calibrated hand ocular. Migration area was calculated from the mean diameter. The bottom of the well was not included in the migration area. The migration index (MI) was calculated by dividing the mean of the migration of cells incubated with each supernatant from stimulated cultures (or in the direct LMAT with each spleen suspension) by the mean area of the migration of cells incubated in the corresponding supernatant from control cultures (or in the direct LMAT of cell suspension without spleen suspension). Results are shown as migration index $\pm$ standard deviation of the mean.

RESULTS

LMAT. Triplicate and quadruplicates varied less than 5% in both direct and indirect LMAT. Leukocytes from three sarcoid patients were tested with different spleen suspensions in the direct LMAT. Migration indexes were 0.97 ± 0.09 (mean $\pm$ SD) for normal spleen suspension, 0.91 ± 0.10 for Hodgkin's spleen suspension, and 0.91 ± 0.11 for sarcoid spleen suspension. One of these patients was tested in both direct and indirect assay, and no differences were found in MI values.

Because of the higher sensitivity of the indirect LMAT (Anders & Natvig, 1976; Clausen, 1973), 22 additional patients were studied using this assay. The results are shown in Table 1. There is no

TABLE 1. Response of lymphocytes from sarcoid patients to different spleen suspensions*

Suspension used	LMAT†		Lymphocyte transformation	
	No. of patients	Migration index	No. of patients	Stimulation index
Reconstituted normal spleen	6	0.89 ± 0.08		
Normal spleen	22	0.87 ± 0.12	22	1.14 ± 0.42
Reconstituted Hodgkins' spleen	6	0.95 ± 0.11		
Hodgkins' spleen	23	0.90 ± 0.10	21‡	1.08 ± 0.44
Reconstituted sarcoid spleen	6	0.94 ± 0.04		
Sarcoid spleen	23	0.89 ± 0.10	22	1.03 ± 0.48

* Spleen suspension prepared as a standard Kveim preparation of Chase-Siltzbach Type 1, but without heating or preservative.

† Indirect leukocyte migration inhibition test under agarose.

‡ One patient with stimulation index 6.61 was not included in these results.

absolute cut-off point in the migration inhibition test, but in most studies a migration index less than 0.77–0.80 has been taken as a significant response. Migration indexes between 0.76 and 0.79 were found in 5 of 23 patients when sarcoid spleen suspensions were used, and in one patient the MI was 0.73. However, when the migration indexes were calculated by dividing the migration area of the test

supernatant by that of a control supernatant reconstituted with sarcoid spleen material, indexes of 0.80–0.88 were found for all four cases in which the reconstitution was made. For two further patients, when the supernatant was not reconstituted with sarcoid spleen material, migration indexes were 0.78 and 0.77; in the latter patient MI to normal spleen and Hodgkin's spleen materials was also 0.77. Therefore, it can be concluded that no inhibition of leukocyte migration under agarose was found either in direct or indirect LMAT to sarcoid spleen material.

Migration index less than 0.80 to normal spleen material was found in 5 of 22 patients with sarcoidosis (0.71–0.79). However, when control supernatants reconstituted with normal spleen material were used, migration indexes were 0.89 (vs 0.79), 0.91 (vs 0.67), and 0.76 (vs 0.71). In two cases in which reconstitution was not made, migration indexes were 0.78 and 0.77; in the latter patient MI to sarcoid spleen and Hodgkin's spleen materials was also the same.

Migration indexes less than 0.80 to Hodgkin's spleen suspension were found in 4 of 23 patients. When the MI was calculated using reconstituted control supernatant, only two patients showed MI of 0.77, one of those being the same patient who showed an MI of 0.77 in tests using sarcoid and normal spleen suspensions. In summary, no significant inhibition of migration under agarose was found with normal or Hodgkin's spleen suspensions.

Lymphocyte ³H-thymidine incorporation. In this assay, a two- or threefold increase in the response to antigen as compared with control cultures has generally been considered significant. Hodgkin's spleen material induced a stimulation index of 6.61 in one patient. The next highest stimulation index to spleen suspensions was 2.24, which was to sarcoid material. However, the same patient had a stimulation index of 2.05 to normal spleen suspension. Only one additional patient had a response with stimulation index greater than 2 (SI 2.14 to Hodgkin's spleen suspension). If a threefold response (Oppenheim *et al.*, 1975) is taken as a significant response, no significant lymphocyte DNA synthesis *in vitro* was produced by lymphocytes of 22 patients with sarcoidosis by normal or sarcoid spleen suspensions. One of 22 patients responded in this assay to Hodgkin's spleen material.

DISCUSSION

A handicap in using Kveim tissue suspension for skin testing is the long time required for the papule to mature at the injection site (4–6 weeks). Therefore, several attempts have been made to develop specific *in vitro* tests for sarcoidosis. In some studies involving few patients some increases in morphological lymphocyte transformation in the presence of Kveim material have been reported when the response was measured as the percentage of either large cells or mitoses (Hirschhorn *et al.*, 1964) or blasts (Siltzbach *et al.*, 1969; Mankiewicz, Kurti & Beaud, 1971). Cowling *et al.* (1964), measuring the response as the percentage of blasts, could not find any response to Kveim material. In studies based on isotope incorporation into lymphocyte DNA, no significant response was noted (Siltzbach *et al.*, 1969; Topilsky *et al.*, 1972; Izumi, Nilsson & Ripe, 1973), except in one study (Zweiman & Israel, 1976) in which at least one Kveim preparation in 14 of 45 sarcoid patients and in 4 of the 30 controls induced transformation of lymphocytes, considering a stimulation index greater than 2 as a significant response. In a previous study, using three different Kveim materials *in vitro*, one of us found no lymphocyte transformation in 31 Kveim skin test positive patients with sarcoidosis (Horsmanheimo, 1973).

Contradictory findings have been reported for the migration inhibition test in sarcoid patients. Inhibition of migration was observed by some investigators (Zweiman & Israel, 1976; Hardt & Wansstrup, 1969; Becker *et al.*, 1972; Jones Williams *et al.*, 1972), whereas negative results have been reported by Topilsky *et al.* (1972) Hardt *et al.* (1976), and Rocklin *et al.* (1972). Some Kveim materials

have been reported to inhibit macrophage migration in diseases other than sarcoidosis (Willoughby & Mitchell, 1971; Brostoff & Walker, 1971; Pagaltso *et al.*, 1971).

The LMAT has been used for studying Kveim reactivity *in vitro* only as a direct assay. The ability of sarcoid lymphocytes to release LIF in direct LMAT has been studied by Zweiman & Israel (1976) using different Kveim materials. They found significant inhibition more often in sarcoid patients than in controls. However, very small differences were found and there was considerable overlap. Hardt *et al.* (1976), using Danish Kveim material, found no inhibition of leukocyte migration in the direct LMAT in 46 sarcoid patients. Indirect LMAT, in which the ability of lymphocytes to release LIF in cultures is tested, has been shown to be more sensitive than the direct LMAT (Anders & Natvig, 1976; Clausen, 1973). In addition, the indirect LMAT has also been shown to correlate with lymphocyte transformation induced by the same antigens (Anders & Natvig, 1976). Therefore, these tests were used in parallel in the present study. The failure of Kveim material to induce a significant lymphocyte DNA synthesis response confirms previous studies (Siltzbach *et al.*, 1969; Cowling, Quaglino & Barrett, 1964; Topilsky, 1972; Izumi, Nilsson & Ripe, 1973; Horsmanheim, 1973). For the few patients tested by direct LMAT in the present study, no inhibition of leukocyte migration was seen, confirming the results of Hardt *et al.* (1976) and, in part, those of Zweiman & Israel (1976). In addition, in the indirect LMAT no Kveim-induced LIF release was found in 22 patients with sarcoidosis. Thus, the results of the present study, in which 3 different *in vitro* tests of cell-mediated immunity were used to study Kveim reactivity *in vitro*, strongly suggests that the Kveim reaction is not an expression of cell-mediated immunity.

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REFERENCES

- ANDERS, E.M. & NATVIG, J.B. (1976) Cell-mediated immunity to viruses measured by the indirect agarose technique of leukocyte migration inhibition. *Cell Immunology*, **27**, 214.
- ASTOR, S.H., SPITLER, L.E., FRICK, O.L. & FUDENBERG, H.H. (1973) Human leukocyte migration inhibition in agarose using four antigens: Correlation with skin reactivity. *Journal of Immunology*, **110**, 1174.
- BECKER, F.W., DEICHER, H., KRULL, P. & KALDEN, J.R. (1972) Leukocyte migration test in sarcoidosis. *Lancet*, **i**, 120.
- BERGSTRAND, H., KALLEN, B. & NILSSON, O. (1974) On the reactivity of human leukocytes to PPD in Clausen's agarose migration technique. *Acta allergologica*, (Kbh), **29**, 117.
- BROSTOFF, J. & WALKER, J.G. (1971) Leukocyte migration inhibition with Kveim antigen in Crohn's disease. *Clinical and Experimental Immunology*, **9**, 707.
- CLAUSEN, J.E. (1971) Tuberculin-induced migration inhibition of human peripheral leukocytes in agarose medium. *Acta allergologica*, (Kbh), **26**, 56.
- CLAUSEN, J.E. (1973) Migration inhibitory effect of cell-free supernatants from tuberculin-stimulated cultures of human mononuclear leukocytes demonstrated by two-step MIF agarose assay. *Journal of Immunology*, **110**, 546.
- COWLING, D.C., QUAGLINO, D. & BARRETT, P.K.M. (1964) Effect of Kveim antigen and old tuberculin on lymphocytes in cultures from sarcoid patients. *British Medical Journal*, **i**, 1481.
- ERARD, P. (1974) Technical study of the leukocyte migration inhibition test in agarose. Application to PPD and to hepatitis B antigen. *Clinical and Experimental Immunology*, **18**, 439.
- FLEER, A., VAN DER HART, M., BLOK-SCHUT, B.J.T. & SCHELLEKENS, P.T.A. (1976) Correlation of PPD and BCG-induced leukocyte migration inhibition, delayed cutaneous hypersensitivity, lymphocyte transformation *in vitro* and humoral antibodies to PPD in man. *European Journal of Immunology*, **6**, 163.
- FREI, P.C., ERARD, P. & ZINKERNAGEL, R. (1973) Cell-mediated immunity to hepatitis-associated antigen (HAA) demonstrated by leukocyte migration test during and after acute B hepatitis. *Biomedicine*, **19**, 379.

- HARDT, F., VEIEN, N., BENDIXEN, G., BRODTHAGEN, H., FARBER, V., GENNER, J., HECKSCHER, T., RINGSTED, J., FREIESLEBEN SØRENSEN, S., WANSTRUP, J. & WIE, A. (1976) Immunological studies in sarcoidosis. A comparison of *in vivo* and *in vitro* Kveim tests. *Annals of the New York Academy of Science*, **278**, 711.
- HARDT, F. & WANSTRUP, J. (1969) Sarcoidosis. An *in vitro* Kveim reaction based on the leukocyte migration test. *Acta pathologica et microbiologica Scandinavica*, **76**, 493.
- HIRSCHHORN, K., SCHREIBMAN, R.K., BACH, F.H. & SILTZBACH, L.E. (1964) *In vitro* studies of lymphocytes from patients with sarcoidosis and lymphoproliferative diseases. *Lancet*, **ii**, 842.
- HOFFMAN, P.M., SPITLER, L.E., HSU, M. & FUDENBERG, H. (1975) Leukocyte migration inhibition in agarose. *Cellular Immunology*, **18**, 21.
- HOFFMAN, P.M., SPITLER, L.E. & HSU, M. (1976) Leukocyte migration inhibition in guinea-pigs. I. Correlation with skin test reactivity and macrophage migration inhibition. *Cellular Immunology*, **21**, 358.
- HORSMANHEIMO, M. (1973) Lymphocyte transformation and the Kveim test. *Lancet*, **i**, 1120.
- HORSMANHEIMO, M., HORSMANHEIMO, A., BANOV, C.F., AINSWORTH, S.K. & FUDENBERG, H.H. (1977) Cell-mediated immunity *in vitro* in atopic dermatitis. *Archives of Dermatology*, in press.
- IZUMI, T., NILSSON, B.S. & RIPE, E. (1973) *In vitro* lymphocyte reactivity to different Kveim preparations in patients with sarcoidosis. *Scandinavian Journal of Respiratory Disease*, **54**, 123.
- JONES WILLIAMS, W., PIOLI, E., JONES, D.J. & DIGHEHO, M. (1972) The Kmif (Kveim-induced macrophage migration inhibition factor) test in sarcoidosis. *Journal of Clinical Pathology*, **25**, 951.
- KOONAKOSIT, R. & PETCHCLAI, B. (1975) Leukocyte migration test in a modified agarose medium. *American Journal of Clinical Pathology*, **64**, 531.
- LOMNITZER, R., RABSON, A.R. & KOORNHOF, H.J. (1975) Production of leukocyte inhibitory factor (LIF) and macrophage inhibitory factor (MIF) by PHA-stimulated lymphocytes. *Clinical and Experimental Immunology*, **22**, 522.
- MANKIEWICZ, E., KURTI, V. & BELAND, J. (1971) Diagnostic procedures in sarcoidosis. *Canadian Medical Association Journal*, **104**, 684.
- MYGIND, K. & STENBJERG, S. (1974) Leukocyte migration test in agarose. The use of puromycin in disclosure of non-immunological inhibition. *Acta Pathologica et microbiologica Scandinavica*, Sect. B., **82**, 277.
- OPPENHEIM, J.J., DOUGHERTY, S., CHAN, S.P. & BAKER, J. (1975) Use of lymphocyte transformation to assess clinical disorders. In: *Laboratory Diagnosis of Immunologic Disorders* (Ed. by G.N.Vyas, D.P.Stites and G. Brecher), p. 87. Grune and Stratton, Inc., New York.
- PAGALTOS, A.S., WILLOUGHBY, J.M.T., KUMAN, P.J. & DAWSON, A.M. (1971) *In vitro* inhibition of leukocyte migration by sarcoid spleen suspension in coeliac disease and dermatitis herpetiformis. *Lancet*, **ii**, 1179.
- PASSALEVA, A., FORTI, A. & RICCI, M. (1974) Inhibition of leukocyte migration in man by the agarose plate technique using protein purified derivations (PPD) from three different sources. *Acta allergologica* (Kbh), **29**, 209.
- ROCKLIN, R.E. (1974) Products of activated lymphocytes: Leukocyte inhibitory factor (LIF) distinct from migration inhibitory factor (MIF). *Journal of Immunology*, **112**, 1261.
- ROCKLIN, R.E. (1975) Partial characterization of leukocyte inhibitory factor by concanavalin A-stimulated human lymphocytes (LIF ConA). *Journal of Immunology*, **114**, 1161.
- ROCKLIN, R.E., SHEFFER, A.L. & DAVID, J.K. (1972) Sarcoidosis: A clinical and *in vitro* immunologic study. In: *Proceedings of the Sixth Leukocyte Culture Conference* (Ed. by M.R. Schwarz), p. 743. Academic Press, New York.
- SILTZBACH, L.E. (1967) Sarcoidosis: Clinical features and management. *Medical Clinics of North America*, **51**, 483.
- SILTZBACH, L.E. (1976) Qualities and behaviour of satisfactory Kveim suspensions. *Annals of the New York Academy of Science*, **278**, 665.
- SILTZBACH, L.E., GLADE, P.R., HIRSHAUT, Y., VIEIRA, L.O.B.D., CELIKOGLU, J.S. & HIRSCHHORN, K. (1969) *In vitro* stimulation of peripheral lymphocytes in sarcoidosis. In: *Fifth International Conference on Sarcoidosis* (Ed. by L. Levinsky and F. Macholda), p. 217. State Printing House, Prague.
- TAUTZ, C., KHUEN, F. & AX, W.Z. (1974) Human leukocyte migration under agarose: Migration inhibition by soluble and tumor antigens with special reference to antimealoma activity in healthy blacks. *Zeitschrift für Immunitätsforschung und experimentelle Therapie*, **147**, 155.
- TOPILSKY, M., WILLIAMS, M., SILTZBACH, L.E. & GLADE, P.R. (1972) Lymphocyte response in sarcoidosis. *Lancet*, **i**, 117.
- WILLOUGHBY, J.M.T. & MITCHELL, D.N. (1971) *In vitro* inhibition of leukocyte migration in Crohn's disease by a sarcoid spleen suspension. *British Medical Journal*, **iii**, 155.

Toxic epidermal necrolysis

FIVE CASE REPORTS OF UNUSUAL PRESENTATION IN ZAMBIANS

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SUMMARY

A new clinical presentation of epidermal necrolytic reaction affecting a limited area is reported in 5 Zambian patients. No definite causative factor could be identified in any of these cases. A staphylococcal aetiology is suspected because of the compatible histological features. However, none of the patients had pyogenic skin lesions and staphylococcus was not isolated from the skin lesions.

Toxic epidermal necrolysis (T.E.N.) is now widely accepted as a pattern of cutaneous reaction. Although the reaction is said to be multifactorial, so far only staphylococcal infection and drug hypersensitivity have been proved sufficiently beyond doubt. Animal models have been successfully devised for both (Wuepper, Dimond & Kuutson, 1975). Apart from the cases due to these two known causative factors, there have been many other cases of T.E.N. in which the cause could not be identified. Thus in an extensive review of 128 cases by Lyell in 1967 as many as 34 were considered idiopathic.

During the past one year we have seen a considerable number of patients at the University Teaching Hospital in Lusaka who presented with a localized form of T.E.N. No obvious causative agent could be identified in these cases. The following are the typical case histories of five such patients.

Case 1

A 25-year-old Zambian male complained of a scald like lesion on the chest, which developed overnight. He went to bed as usual and woke up in the night due to burning pain on the right side of his chest. He did not see any insect and he slept again. Early next morning he noticed the lesion. He denied having taken any drug or applied anything topically. There was no history of recent pyogenic or other bacterial infections. There was no exposure to irritant chemicals. A small area on the right side of his chest looked as if it had been scalded (Fig. 1). The skin on the rest of the body was normal. Unfortunately no investigations were done as the lesion was presumed to be a primary irritant reaction due to accidental contact with an unknown chemical agent or a poisonous insect. He was advised to apply framycetin cream to prevent secondary bacterial infection. He was seen after a week without improvement and fresh impetigo-like lesions had appeared at the edge. Cultures were not taken but he improved with penicillin injections over the next 5 days.



FIGURE 1. Case 1. Scaldlike lesion on the chest.

Case 2

A 28-year-old Zambian male had a burning pain on his back which disturbed his sleep during the night and he could not sleep again. In the morning he noticed that the skin on his back was as if scalded. There was no history of contact with irritant chemicals or topical medicines. There was no history of any recent illness and he had not taken drugs for a long time. The entire skin on his back showed a scalding reaction, more severely on the right half. Nikolsky's sign was positive. The skin in other areas was perfectly normal. Blister fluid culture was negative but biopsy was not done. It was feared that the reaction might become generalized and he was put on prednisolone 60 mg daily. Surprisingly the entire lesion had dried up when he was seen next day. It was considered as spontaneous resolution and the steroid was withdrawn over a week.

Case 3

A 15-year-old Zambian female was referred to the dermatology clinic for spontaneous scalding of the face which had been recurring over the previous 9 months. Each time the scalding reaction occurred during the night while the patient was comfortably asleep. She usually noticed the scalding on the following morning. There had been no constitutional upset and the skin always became normal in a week. There had been no history of pyogenic infection of the skin and there was also no association with eye, nasal or throat infections. There was no history of drug ingestion prior to the episodic



FIGURE 2 (a & b). Case 3. Recurring lesions on the face.

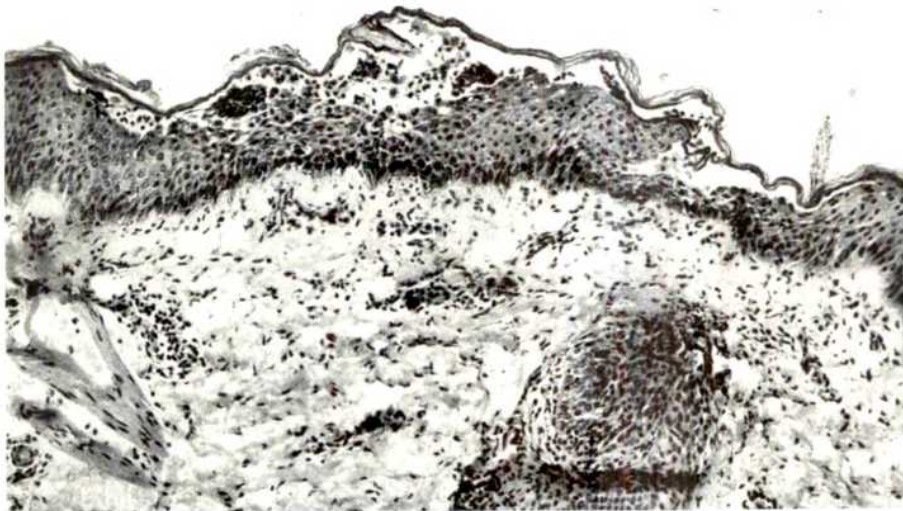


FIGURE 3. Case 3. First episode. Intraepidermal bulla on biopsy.

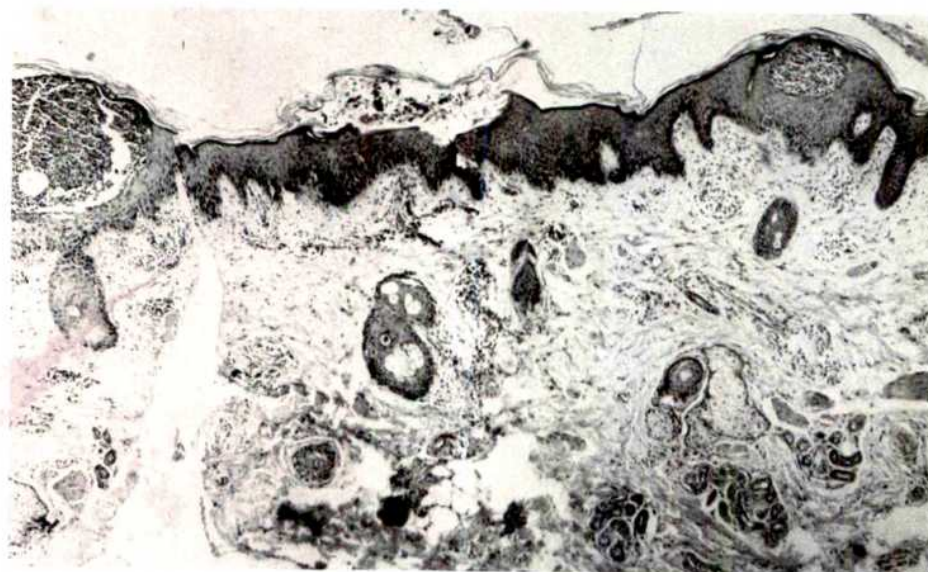


FIGURE 4. Case 3. Second episode. Histological features similar to impetigo.

attacks of T.E.N. She was treated with antibiotics on previous occasions with the diagnosis presumed to be bullous impetigo. According to the patient there had been no change in the duration of the attacks with or without treatment.

She was seen for the first time in the dermatology clinic in December 1976 with a sharply localized scalded appearance of the facial skin (Fig. 2a). There was no evidence of pyogenic infection elsewhere on the body. The eyes, nose and throat were clear. Except for a slight burning sensation the patient was not in discomfort. The blister fluid cultures were negative. A biopsy was taken which revealed subcorneal bullae filled with polymorphs and eosinophils (Fig. 3). She was given penicillin injections, and framycetin cream for topical application. The skin became normal in less than a week.

She was seen for the second time in February 1977 with an identical clinical presentation (Fig. 2b). Again no pyogenic skin lesions were seen and cultures were sterile. Biopsy revealed features similar to the first time but looked more like impetigo (Fig. 4). During this episode, she was given only placebo therapy of vitamin tablets and the condition spontaneously resolved in a week as before.

Case 4

A 20-year-old Zambian male presented with spontaneous scalding of the face which started with a severe burning sensation during the previous night. His sleep was disturbed in the middle of the night but he was not aware of any insect bite. There was no history of exposure to corrosive chemicals and he also denied having taken drugs. There was no history of similar episodes in the past. On examination the entire face presented a mild but uniformly diffuse scalding reaction without obvious bulla formation. Nikolsky's sign was positive. There were no pyogenic lesions on the body. Eyes, nose and throat were clear. The blister fluid was clear and microscopy and culture were negative. Biopsy showed an extensive intra-epidermal split below the granular layer throughout the section (Fig. 5). The condition resolved spontaneously without treatment in a week.

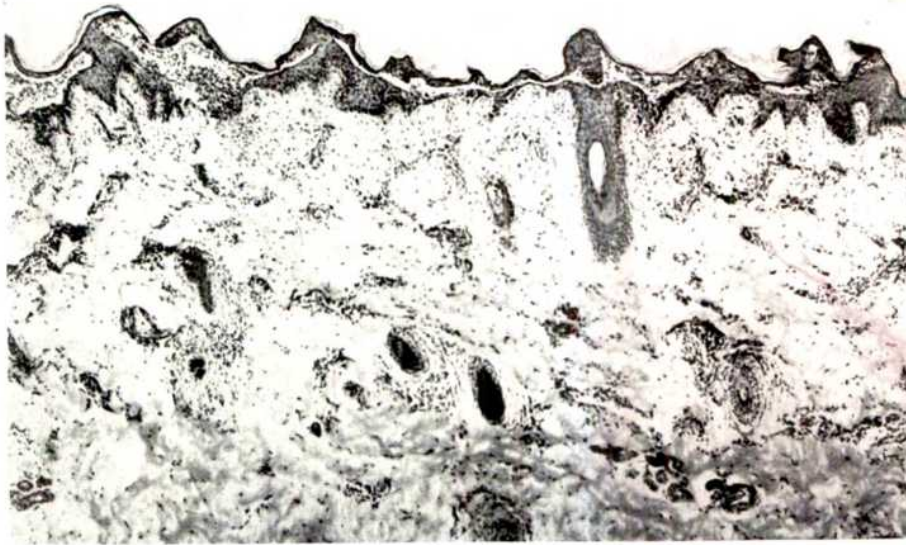


FIGURE 5. Case 4. Biopsy showing extensive intra-epidermal cleavage.

Case 5

A 16-year-old Zambian female presented with spontaneous blisters on the face which developed during the previous night while she was asleep. The face was swollen and she was unable to open her eyes. The scalding reaction was more severe than in the other cases with large, clear blisters on the face. Microscopy was negative and so was the culture of the blister fluid. The history was non-contributory as in the other cases. There were no signs of pyogenic skin infection and throat and nose were clear. Biopsy was done and histology showed slight variation from the other two cases. The intra-epidermal bullae were focal around the follicular openings and extended along into the mid-dermis with marked cellular reaction. Most of the cells were polymorphs.

The patient was put on placebo therapy but was lost to follow up. The eruption was presumed to have cleared spontaneously as in the other patients.

DISCUSSION

The clinical picture was almost identical in all these five cases. The peculiar lesion had developed suddenly within 6–8 h during the night with relatively little discomfort to the patient. Two of them noticed the skin lesion in the morning having slept well. The lesion was localized to a limited area and in three cases the face was affected. One female patient suffered many identical recurrences. Histologically all the cases have shown intraepidermal bulla formation associated with mild to severe inflammation. In some of the cases the bulla formation was focal with a predominant follicular involvement. But in some others there was an extensive cleavage throughout the epidermis (Fig. 5). Spongiosis was not a feature of bulla formation in any of the biopsies. Spontaneous resolution was observed in all the cases.

When the first few cases were seen, we considered them as non-specific reactions but with increasing experience a common pattern was recognized and we investigated the subsequent cases for possible common aetiological factors. The sudden onset of the clinical lesion with a burning sensation

and its localization initially gave us a strong suspicion of external agents, like chemical irritants in the form of either herbal medicines or cosmetic aids, or accidental contact with vesicant insects or even insect bites. None of the patients admitted having applied anything on their skin before the lesion appeared. We have probed into the daily routine of all these patients but failed to discover any possible contact with any primary irritants. Insect bites and contact with vesicant beetles although suspected were dismissed, again since none of the patients noticed them and also in such cases the clinical picture would have been different with linear and bizarre patterns of blisters. The symmetrical facial lesions as seen in three of our patients did not give such an impression. Moreover primary irritants and insect reactions usually produce spongiotic bulla formation. (Exceptionally, petrol, paraffin and some plant juices cause 'scalds' with intra-epidermal splitting of the skin (Lyell, personal communication).) This line of thinking leads us to believe that these reactions were not caused by external agents. Drugs are known to produce localized T.E.N. occasionally, but an absolutely negative history of drug ingestion and the histological features in our cases are strong points against such speculation. Staphylococcal T.E.N. is commonly seen in children below the age of 10 years, but it has been reported in adults (Rothenberg *et al.*, 1973). Limited involvement is also known to occur in children. Samuels (1967) reported 10–50% of skin involvement in his series of 42 cases although a common localization to the face has not been mentioned. Since staphylococcus was not isolated in our cases, speculation on this aetiology has to depend largely on the compatibility of the histological features.

In the earlier reviews of staphylococcal T.E.N., histological features have not been considered essential for the diagnosis. Lyell mentioned only few biopsies out of 30 cases and Samuels described three biopsies in his series of 42 cases of staphylococcal T.E.N. Similarly only two biopsies were done in seven cases reported by Lyell, Dick & Alexander (1967). However, the majority of these patients had a history of pyogenic infections and staphylococcus was readily isolated except in a few. Positive diagnosis was made on those two points and not on histology which at that time was not clearly delineated. Now it is well established that staphylococcal T.E.N. produces an intra-epidermal split at the level of the granular cell layer with superficial epidermal loss, whereas the drug induced form is characterized by subepidermal bulla formation with entire or nearly entire epidermal detachment (Lever, 1975). Further to stress the diagnostic importance of the histology in staphylococcal T.E.N., it was even stated that mere recovery of the appropriate strain of the organism was insufficient evidence to document the diagnosis (Malkinson & Pearson, 1974). This statement apparently undermined the importance of isolating the organism which we consider is equally essential. However, we feel that consistent intra-epidermal bulla formation in all our cases is sufficient reason to suspect a staphylococcal aetiology. Lyell also suspected an unidentified staphylococcal infection in 22 children in the idiopathic group. It is also pertinent to mention here that 25% of all new cases seen in our clinic are pyogenic infections and classical staphylococcal T.E.N. in infants and children is not an uncommon disease. Bullous impetigo lesions are sometimes associated with a limited T.E.N. in the surrounding skin. So with this background of abundant staphylococcal infections, we feel that the possibility of staphylococcal infection producing a limited form of T.E.N. should be excluded beyond all reasonable doubt before other speculations can be entertained.

A prospective study with a systematic search for staphylococcal infections in the subsequent cases is underway.

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REFERENCES

- LYELL, A. (1967) A review of toxic epidermal necrolysis in Britain. *British Journal of Dermatology*, **79**, 662.
- LYELL, A., DICK, H.M. & ALEXANDER, J.O.D. (1969) Out-break of toxic epidermal necrolysis associated with staphylococci. *Lancet*, **i**, 787.
- LEVER, W.F. & LEVER, G.S. (1975) In: *Histopathology of the Skin*, 5th edn, pp. 270-271. J.B.Lippincott Company, Philadelphia.
- MALKINSON, F.D. & PEARSON, R.W. (1974) Vesicular and bullous disease In: *Year Book of Dermatology*, p. 212. Year Book Medical Publishers, Chicago.
- ROTHENBERG, R., RENNA, F.S., DREW, T.M. & FLENGOLD, D.S. (1973) Staphylococcic scalded skin syndrome in an adult. *Archives of Dermatology*, **108**, 408.
- SAMUELS, M.J. (1967) Toxic epidermal necrolysis. A report of 42 cases. *British Journal of Dermatology*, **79**, 762.
- WUEPPER, K.D., DIMOND, R.L. & KNUTSON, D.D. (1975) Studies of the mechanism of epidermal injury by a staphylococcal epidermolytic toxin. *Journal of Investigative Dermatology*, **65**, 191.

Possible defects in triacylglycerol and phosphatidylcholine metabolism in psoriatic epidermis

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SUMMARY

Biopsies of normal skin, psoriatic lesions and the adjacent psoriatic skin were incubated with 1-¹⁴C-acetate, ³²P-orthophosphate and U-¹⁴C-glycerol.

Total incorporation of 1-¹⁴C-acetate into psoriatic lesions (17 samples) was 50% higher than into the adjacent uninvolved epidermis (9 samples) and 120% higher than into normal epidermis (10 samples). In the psoriatic lesion a much higher proportion of the total incorporation was into the neutral lipids and was due mainly to a very high incorporation of 1-¹⁴C-acetate into the triacylglycerols. The 1-¹⁴C-acetate incorporated into the phospholipids and especially phosphatidylcholine was proportionally much less in the psoriatic lesion and uninvolved psoriatic epidermis than in normal epidermis even though the incorporation of ³²P-orthophosphate and U-¹⁴C-glycerol, both representing *de novo* synthesis, into the phospholipids in the psoriatic lesions and uninvolved epidermis were higher (four-fold in lesion) than in normal epidermis.

Our findings (1) are evidence for a much increased triacylglycerol synthesis in psoriatic epidermis which would account for the long-known observation of lipid droplet accumulation in psoriatic cells; (2) suggest that in psoriatic epidermis there is a defect in phospholipid metabolism mainly involving phosphatidylcholine and the deacylation (phospholipase A)-reacylation (phospholipid acyltransferase) cycle for fatty acid transfer.

Historically, reported changes in skin lipids have long been associated with the psoriatic condition (Mier & Cotton, 1976). Unfortunately, these changes have often been poorly defined and the experimental approach open to criticism, with the result that no general agreement has been reached on the state of lipid metabolism in psoriatic epidermis. Recently, however, Cooper *et al.* (1976) have provided convincing evidence from incorporation studies with (U-¹⁴C)-glucose of increased sterol synthesis in both lesion and uninvolved psoriatic epidermis compared with normal epidermis.

In previous reports from our laboratory (Long, 1976, 1977), it was shown that patterns of *in vitro* incorporation of 1-¹⁴C-acetate and ³²P-orthophosphate into the lipids of epidermal cell suspensions and epidermal slices were consistently reproducible under controlled conditions. The ability to obtain a 'radioactive lipid profile' which is characteristic of the lipid metabolism in the particular tissue has

made it possible to compare in detail some aspects of the metabolism of neutral and polar lipids in normal and in psoriatic tissue *in vitro* and the results are presented in this paper.

MATERIALS AND METHODS

Skin biopsies

The skin was first cleaned with Savlodil and then with methylated spirit BPC and wiped dry. Lignocaine 2% without adrenaline was used as local anaesthetic and a zone of skin delineated with a 10 mm diameter punch. An epidermal biopsy (Arthur & Shelley, 1959) of this area was taken using a stainless steel blade (Paragon number 17).

Biopsies were taken from untreated lesions in psoriatic patients who exhibited chronic stable plaques, erupting guttate lesions or resolving expressions of their disease; and from adjacent (approximately 2 cm from lesion) clinically uninvolved skin. Areas biopsied were either on the upper arm or on the outer aspects of the thigh. Control skin specimens were obtained from these same sites in healthy volunteers and from patients who had minor dermatological lesions (e.g. common warts) but were otherwise normal.

Incubation of skin slices with $1\text{-}^{14}\text{C}$ -acetate and/or ^{32}P -orthophosphate

Each biopsy specimen was rinsed in 0.9% sodium chloride and transferred to a bijou bottle containing Krebs-Ringer bicarbonate (0.49 ml), 0.3 M glucose (0.05 ml) and 29 μCi of $1\text{-}^{14}\text{C}$ -acetate and/or 30 μCi ^{32}P -orthophosphate (sodium $1\text{-}^{14}\text{C}$ -acetate, 59 mCi/mM and ^{32}P -orthophosphate, 60 Ci/mg P; Radiochemical Centre, Amersham). In addition a few slices were incubated with ^{32}P -orthophosphate (30 μCi) and U- ^{14}C -glycerol (25 μCi) (Radiochemical Centre, Amersham, 49 mCi/mM). Incubations were carried out as described by Summerly & Woodbury (1971) at 37°C for 22 h. After culture the skin slices were rinsed with 0.9% sodium chloride and placed in 1 M calcium chloride for between 1 and 2 h at room temperature after which time the small amount of papillary dermis could be peeled from the under-surface of the epidermis using a dissecting microscope. Each disc of epidermis was rinsed in 0.9% sodium chloride, air-dried and weighed on an electrobalance.

Extraction of lipids from epidermal specimens

The epidermal disc was homogenized in a small Potter glass tissue grinder and the lipids were extracted with chloroform-methanol (2:1, v/v) and the residue from this extraction washed twice with chloroform-methanol (2:1, v/v). The total lipid extract (5.0 ml) was washed with 0.1 M sodium chloride according to Folch *et al.* (1957) and the chloroform was evaporated by a stream of N_2 . The lipid residue was dissolved in dry chloroform and stored at -20°C until required.

Fractionation of lipids

(a) *Column chromatography.* Total lipids were separated by chromatography on a small column of silica gel H (250 mg, Merck, Darmstadt, W. Germany) into (a) neutral lipids, (b) acidic phospholipids and glycolipids, and (c) choline-containing phospholipids as described by Gray (1967).

(b) *Thin-layer chromatography.* Precoated thin-layer plates (20 × 20 cm) of silica gel 60 (Merck, Darmstadt, W. Germany) were used without activation. Neutral lipids, phospholipids and glycolipids were separated into individual components by the procedures described previously (Gray & Yardley, 1975). Radioactive lipids were identified by co-chromatography with reference lipids (Long, 1976) which were visualized by exposure to iodine vapour.

Measurement of radioactivity

The radioactive components were detected on the thin-layer plates by autoradiography and each component was recovered and its radioactivity measured as described by Long (1976). Radioactivity was measured with a Packard Tri-carb liquid scintillation spectrophotometer 2425 and was expressed as counts/s/epidermal disc (area = 78.5 mm) and as counts/s/mg weight of epidermis. Both expressions of results are given in recognition of the obvious morphological differences that exist between normal and psoriatic epidermis.

RESULTS

Incorporation of $1-^{14}\text{C}$ -acetate into epidermal lipids

Initially we investigated the incorporation of $1-^{14}\text{C}$ -acetate (and ^{32}P -orthophosphate) into epidermal lipids after both 4 and 22 h incubation periods. Differences in the patterns of incorporation into lipids in normal epidermis compared with psoriatic epidermis which were apparent after a 4 h incubation were enhanced further after 22 h. The much higher level of radioactivity in the lipids after 22 h enabled the incorporation into minor lipid components to be measured with less error. Thus the 22 h incubation period was subsequently chosen for all the work described in this paper.

The total incorporation of $1-^{14}\text{C}$ -acetate (per epidermal disc) in the psoriatic lesion (Table 1) was approximately 50% higher than in the adjacent uninvolved epidermis and about 120% higher than in normal epidermis. Compared with the normal epidermis a much higher proportion of $1-^{14}\text{C}$ -acetate was incorporated into the neutral lipids in both the psoriatic lesion and the adjacent uninvolved epidermis and a corresponding lower proportion into the phospholipids. The proportion of $1-^{14}\text{C}$ -acetate incorporated into the glycolipids in both normal and psoriatic epidermis was not significantly different. In absolute terms the acetate labelling of all neutral lipids in both the psoriatic lesion and the adjacent uninvolved epidermis was much higher than in the normal epidermis (Table 2). The largest increase in acetate incorporation occurred in the triacylglycerols from the psoriatic lesion and from the adjacent uninvolved epidermis (Tables 2 and 3) and compared with that incorporated into the triacylglycerols from normal epidermis, they accounted for a much larger proportion of the total acetate incorporation. The proportions of $1-^{14}\text{C}$ -acetate into other neutral lipids were not significantly different from those in normal epidermis. The smaller proportion of the total acetate label that was incorporated into the phospholipids in the psoriatic lesion compared with that into the phospholipids in the normal epidermis was due, not only to the large increase in incorporation into the triacylglycerols, but also to the relatively much lower incorporation into the major phospholipid, phosphatidylcholine (Tables 4 and 5). To some extent there was a proportionally lower acetate incorporation into all of the phospholipids in the psoriatic lesion compared with the normal skin (Table 4).

The total incorporation of acetate into the phospholipids of the psoriatic lesion (measured as counts/s/disc ≈ 3400) was similar to that into the phospholipids of the adjacent uninvolved epidermis (≈ 3600) and the normal epidermis (≈ 3400). If measured as counts/s/mg epidermis the incorporation into the phospholipids of the psoriatic lesion (≈ 283) was less than into the phospholipids of normal epidermis (≈ 458).

Incorporation of ^{32}P -orthophosphate into epidermal phospholipids

The patterns of ^{32}P -orthophosphate incorporation into the phospholipids of psoriatic epidermis and normal epidermis (Table 6) were similar. Fifty-seven percent of the total ^{32}P uptake into the epidermal phospholipids was into phosphatidylcholine in both psoriatic and normal epidermis (Table 6) although the total incorporation into the psoriatic lesion (740 ± 106 counts/s/disc) was nearly four-fold that into normal epidermis (192 ± 44 counts/s/disc). The pattern in adjacent uninvolved psoriatic

TABLE 1. *In vitro* incorporation of $1-^{14}\text{C}$ -acetate into phospholipids, glycolipids and neutral lipids of psoriatic and normal epidermis

Epidermis	Total incorporation of $1-^{14}\text{C}$ -acetate		% incorporation of $1-^{14}\text{C}$ -acetate		
	Counts/s/disc*	Counts/s/mg	Neutral lipids	Phospholipids	Glycolipids†
Psoriatic lesion	(17)‡ 20,300 ± 3305	1688 ± 322	(17) 68.3 ± 3.8	16.8 ± 2.4	14.9 ± 2.2
Adjacent uninvolved psoriatic	(9) 12,680 ± 2767	1149 ± 241	(12) 56.8 ± 4.9	28.3 ± 4.4	14.9 ± 1.8
Normal	(10) 8963 ± 1297	1100 ± 169	(13) 40.9 ± 4.4	41.5 ± 4.0	17.6 ± 2.1
Psoriatic lesion vs normal	0.01 > P	n.s.§	0.001 > P	0.001 > P	n.s.
Lesion vs adjacent uninvolved	0.02 > P > 0.01	n.s.	n.s.	0.01 > P	n.s.

* 10 mm diam.; area, 78.5 mm².

† Includes 'polar' ceramide fraction.

‡ Figures in parentheses are numbers of samples.

§ Not significant.

Results expressed as mean ± standard error of the mean.

TABLE 2. Pattern of incorporation of $1-^{14}\text{C}$ -acetate into neutral lipids of psoriatic and normal epidermis

	$1-^{14}\text{C}$ -acetate incorporation as % of total incorporation into epidermal disc		
	Normal epidermis (13)	Psoriatic lesion (17)	Adjacent uninvolved epidermis (12)
Cholesterol	10.2 ± 0.9	17.2 ± 1.1	15.3 ± 1.0
Diacylglycerol	4.7 ± 0.4	6.7 ± 0.6	6.7 ± 0.7
Triacylglycerol	5.0 ± 0.6	19.3 ± 1.4	10.4 ± 1.2
Fatty acids	7.6 ± 0.8	7.4 ± 0.5	10.1 ± 1.0
Sterol ester/squalene	1.0 ± 0.2	0.8 ± 0.1	0.9 ± 0.1
Monoacylglycerol/'non polar' ceramide*	10.7 ± 0.8	17.2 ± 1.1	15.3 ± 1.0
Unidentified	1.8 ± 0.6	1.8 ± 0.9	1.5 ± 0.1
Total $1-^{14}\text{C}$ -acetate incorporation, counts/s/disc	3665 ± 394 (10)	13864 ± 771 (17)	7202 ± 621 (9)

* These compounds were not separated by the thin-layer chromatographic system and remained at the origin of the thin-layer plate.

epidermis was similar and the total incorporation approximately twice that into normal epidermis. The proportions of the total ^{32}P incorporated into phosphatidylcholine, phosphatidic acid, phosphatidyl-(N-acyl)-ethanolamine, phosphatidylserine and monoacylphosphatidylcholine in both normal and psoriatic epidermis were higher than the equivalent proportions in which these phospholipids had been detected by Gray & Yardley (1975) in normal epidermal cells (Table 6). Conversely, the incorporations of ^{32}P into sphingomyelin, diphosphatidylglycerol, phosphatidylinositol and especially phosphatidylethanolamine were much lower relative to their concentration in the epidermal cells.

TABLE 3. Incorporation of $1-^{14}\text{C}$ -acetate into triacylglycerol in psoriatic and normal epidermis

Epidermis	Counts/s/disc	Counts/s/mg
Psoriatic lesion	3424 ± 708 (13)*	264 ± 46 (13)
Adjacent uninvolved	941 ± 433 (8)	79 ± 34 (8)
Normal	428 ± 102 (9)	42 ± 9 (9)
Psoriatic lesion vs control	$0.01 > P$	$0.001 > P$
Psoriatic lesion vs uninvolved	$0.01 > P$	$0.01 > P$
Uninvolved vs control	n.s.	n.s.

* Figures in parentheses are numbers of samples.

TABLE 4. Pattern of incorporation of $1-^{14}\text{C}$ -acetate into the polar lipids of psoriatic and normal epidermis

	$1-^{14}\text{C}$ -acetate incorporation as % of incorporation into total lipids in epidermal disc		
	Normal epidermis (13)	Psoriatic lesion (17)	Adjacent uninvolved epidermis (12)
Diphosphatidylglycerol	0.59 ± 0.23	0.09 ± 0.02	0.22 ± 0.09
Phosphatidic acid	0.59 ± 0.03	0.44 ± 0.16	0.73 ± 0.43
Phosphatidylethanolamine	1.65 ± 0.35	0.68 ± 0.12	1.04 ± 0.26
Phosphatidyl-(N-acyl)-ethanolamine	1.36 ± 0.40	1.09 ± 0.19	1.90 ± 0.47
Phosphatidylserine	2.00 ± 0.53	0.72 ± 0.19	1.73 ± 0.52
Phosphatidylinositol	1.24 ± 0.23	0.59 ± 0.12	0.91 ± 0.17
Phosphatidylcholine	20.70 ± 1.20	6.03 ± 0.90	10.80 ± 1.90
Sphingomyelin	5.30 ± 0.60	2.18 ± 0.25	3.63 ± 0.64
Monoacylphosphatidylcholine	1.60 ± 0.21	0.37 ± 0.06	0.82 ± 0.17
Glucosylceramide	12.90 ± 1.40	11.26 ± 0.99	11.23 ± 1.38
Ceramide ('polar fraction')	4.25 ± 0.82	3.23 ± 0.38	4.58 ± 1.56
Unidentified	6.80 ± 0.59	4.42 ± 0.68	4.54 ± 0.60

TABLE 5. Incorporation of ^{32}P -orthophosphate and $1-^{14}\text{C}$ -acetate into phosphatidylcholine in psoriatic epidermis

Epidermis	^{32}P -orthophosphate incorporation		$1-^{14}\text{C}$ -acetate incorporation		*Ratio $\frac{^{14}\text{C}}{^{32}\text{P}}$
	Counts/s/disc	Counts/s/mg	Counts/s/disc	Counts/s/mg	
Psoriatic lesions	422 ± 70 (21)	42 ± 8 (21)	1592 ± 242 (30)	168 ± 31 (30)	4.9 ± 0.6 (17)
Adjacent uninvolved psoriatic epidermis	247 ± 63 (15)	47 ± 14 (15)	1634 ± 344 (20)	298 ± 71 (20)	11.1 ± 2.1 (11)
Normal epidermis	110 ± 26 (10)	18 ± 6 (10)	1383 ± 202 (17)	182 ± 34 (17)	10.6 ± 1.0 (7)
Psoriatic lesion vs normal	$0.001 > P$	$0.05 > P$	n.s.	n.s.	$0.001 > P$
Psoriatic lesion vs uninvolved	$0.01 > P > 0.05$	n.s.	n.s.	$0.10 > P > 0.05$	$0.05 > P$
Uninvolved vs normal	n.s.	n.s.	n.s.	n.s.	

* Ratio of counts/s/disc. Values are only from those experiments in which both $1-^{14}\text{C}$ -acetate and ^{32}P -orthophosphate were incorporated.

TABLE 6. Pattern of incorporation of ^{32}P -orthophosphate into phospholipids in psoriatic and normal epidermis

	Incorporation as a % of total ^{32}P incorporation		Phospholipid composition of normal epidermal cells (4)*
	Normal epidermis (10)	Psoriatic lesion (21)	
Diphosphatidylglycerol	0.5 ± 0.7	1.6 ± 0.4	3.6 ± 0.7
Phosphatidic acid	3.1 ± 0.6	4.6 ± 1.0	2.4 ± 0.8
Phosphatidylethanolamine	4.2 ± 0.8	3.6 ± 0.5	19.1 ± 1.7
Phosphatidyl-(N-acyl) ethanolamine	6.4 ± 2.3	4.8 ± 0.8	2.4 ± 0.8
Phosphatidylserine	7.7 ± 0.7	9.9 ± 1.4	3.8 ± 0.4
Phosphatidylinositol	2.2 ± 0.8	5.7 ± 1.3	9.5 ± 2.3
Phosphatidylcholine	57.2 ± 3.4	57.0 ± 2.3	38.5 ± 2.7
Sphingomyelin	9.6 ± 0.9	8.6 ± 1.0	20.8 ± 2.7
Monoacylphosphatidylcholine	8.9 ± 1.4	4.2 ± 1.1	ND†

* Gray & Yardley (1975) values expressed as a percentage of total phospholipid.

† Not detected.

Only phosphatidylinositol and diphosphatidylglycerol showed increased proportions of ^{32}P incorporation in psoriatic epidermis compared with normal epidermis.

In biopsies that were labelled both with ^{32}P -orthophosphate and $\text{U-}^{14}\text{C}$ -glycerol the pattern of incorporation of the $\text{U-}^{14}\text{C}$ -glycerol into phospholipids was similar to that of the ^{32}P -orthophosphate. There was also a large increase (approximately 20-fold) in the incorporation of $\text{U-}^{14}\text{C}$ -glycerol into the triacylglycerols in the psoriatic lesions compared with normal epidermis.

DISCUSSION

Total $\text{U-}^{14}\text{C}$ -acetate incorporation into psoriatic lesions was, as might be expected from previous studies (Aso *et al.*, 1971; Cooper *et al.*, 1976), higher than into normal epidermis (Table 1). The level of incorporation into adjacent uninvolved psoriatic epidermis was between that into the psoriatic lesion and into the normal epidermis and the values (Table 1) represent a mean of a range in which some were closer to the values for the psoriatic lesion and others to the value for normal epidermis. The patterns of acetate incorporation into the epidermal lipids (Tables 1-3) suggested that much of the increased uptake of acetate in the psoriatic lesion was the result of a disproportionately increased synthesis of triacylglycerol. This view was supported by the massive increase we observed in the incorporation of $\text{U-}^{14}\text{C}$ -glycerol into the triacylglycerols in the psoriatic lesion. It has long been known (Brody, 1962) from ultrastructural studies that psoriatic epidermal cells contain many lipid droplets. These droplets are similar to the triacylglycerol droplets commonly present in cultured cells (Bensch *et al.*, 1961) and which act as a storage form for exogenous fatty acids. It is reasonably certain that the droplets in psoriatic cells are also triacylglycerols but it is not known whether their formation and accumulation is the result of a much greater synthesis of fatty acids or due to a much stimulated diglyceride acyltransferase (Fig. 1) in the psoriatic epidermal cell as compared with the normal epidermal cell. Accumulation of triacylglycerols may well arise as a secondary response of the cell to an underlying injury (Rees, 1964) and this could be the situation in the psoriatic epidermal cell.

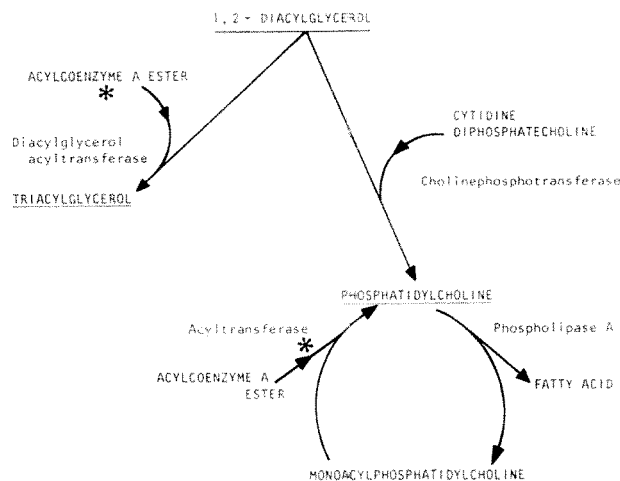


FIGURE 1. The relationship between triacylglycerol and phospholipid metabolism in the mammalian cell. (*) Pathways of lipid metabolism that may be defective in psoriatic epidermis.

However, whether the accumulation of lipid droplets within the psoriatic cell ever reaches a level at which the concentration of lipid itself can cause cell damage again is not known.

It is possible that only hyperkinetic pathological epidermal cells exhibit triacylglycerol accumulation as we have also found an increase in the incorporation of $1\text{-}^{14}\text{C}$ -acetate into triacylglycerols, similar to that in psoriatic epidermis, in a biopsy from a patient with pityriasis rubra pilaris (unpublished results). But we have found no differences, when compared with normal skin, of $1\text{-}^{14}\text{C}$ -acetate incorporation into either triacylglycerols or phosphatidylcholine in biopsies from four patients with autosomal dominant ichthyosis vulgaris (unpublished results).

A most interesting finding was that the incorporation of $1\text{-}^{14}\text{C}$ -acetate into the phospholipids in a psoriatic lesion was proportionally much lower than in normal epidermis. This appeared to be mainly the result of no change, in absolute counts, in the incorporation of $1\text{-}^{14}\text{C}$ -acetate into the major phospholipid, phosphatidylcholine (Tables 4 and 5), in the psoriatic lesion compared with normal skin. Relative to the incorporation into the total phospholipids this represented a significant decrease in the $1\text{-}^{14}\text{C}$ -acetate incorporated into the phosphatidylcholine in the psoriatic lesion ($47.3\% \pm 2.8$ of total, $n = 26$) compared with that in normal epidermis ($59.2\% \pm 2.7$ of total, $n = 13$; lesion vs normal, $0.02 > P > 0.01$). Taken together, the increased synthesis of triacylglycerol and the proportionally decreased acetate uptake into phosphatidylcholine and, to a lesser extent, other phospholipids suggested that the synthesizing systems for triacylglycerol and phospholipids in a psoriatic lesion were in unequal competition for a limited amount of the common precursor, 1,2-diacylglycerol (Fig. 1). However, the much higher incorporation of ^{32}P into the phospholipids of psoriatic epidermis compared with normal epidermis and with adjacent uninvolved psoriatic epidermis indicated that this was not so. The increased incorporations of ^{32}P -orthophosphate and $\text{U-}^{14}\text{C}$ -glycerol, both of which represent *de novo* synthesis of the phospholipid molecule as a whole, showed that there was no lack of precursor diacylglycerol in psoriatic epidermis. Thus the lower acetate incorporation must relate to a decrease in the turnover of the fatty acids in the phospholipid molecules especially those of phosphatidylcholine. Fatty acid turnover in phospholipids usually depends on a deacylation-reacylation cycle (Fig. 1) involving two enzymes, a phospholipase A (A_1 and/or A_2) and an acyltransferase (Lands & Merkl, 1967) and there are a number of possible reasons for the decrease in fatty acid turnover in the phosphatidylcholine. (1) The activity of the epidermal phospholipase A (Long, 1975) might be

decreased in the psoriatic lesion. If this was the case it would be expected that the turnover of fatty acids into other phospholipids equally susceptible to phospholipase A attack would also be lower in psoriasis. With the exception of diphosphatidylglycerol (cardiolipin) and phosphatidylserine the differences between acetate incorporation into other phospholipids in psoriatic lesions and in normal epidermis did not appear significant. (2) There might be a decrease in the activity, due either to inadequate synthesis or partial inhibition, of the acyltransferase enzyme which enables acylation of the monoacylphospholipid (Fig. 1) to occur. There is evidence that phospholipid acyltransferases may be a family of enzymes each specific for a particular substrate (Reitz *et al.*, 1969) and thus it is conceivable that there is a common defect in these enzymes in psoriatic epidermis and this is enhanced in the monoacylphosphatidylcholine-specific acyltransferase. (3) Alternatively, acyltransferase activity may be normal but the availability of acylcoenzyme A esters acceptable to the enzyme may be a limiting factor (Brandt & Lands, 1967) in psoriatic epidermis but not in the normal epidermis. Either of the latter possibilities receives some support from the four-fold decrease in the observed values for the incorporation of 1-¹⁴C-acetate into monoacylphosphatidylcholine in the psoriatic lesion compared with that in normal epidermis (Table 4). In normal epidermis monoacylphosphatidylcholine is not usually present in amounts that are detectable by chemical analysis (Gray & Yardley, 1975) but the fact that it showed a relatively high incorporation of both 1-¹⁴C-acetate and ³²P-orthophosphate is in agreement with its role as an active intermediate in the deacylation-reacylation cycle (Fig. 1).

Another possibility, though in our view an improbable one, is that acylcoenzyme A esters are less readily available for the exchange reaction via the deacylation-reacylation cycle because they are being used in the greatly increased synthesis of triacylglycerol in the psoriatic lesion.

Clearly our results leave many questions unanswered but they have indicated an area of phospholipid metabolism in which, in the psoriatic lesion and also in the adjacent uninvolved epidermis, a real defect may exist and by its nature it may have a far-reaching effect on the membrane structures of the psoriatic cell.

REFERENCES

- ARTHUR, R.P. & SHELLEY, W.B. (1959) The epidermal biopsy: its indications and technique. *Archives of Dermatology*, **80**, 95.
- ASO, K., FUJITA, M., OKAZAKI, T. & KATO, T. (1971) Studies in the lipogenesis of psoriatic epidermis. *Japanese Journal of Dermatology, Series B*, **81**, 161.
- BENSCH, K.G., KING, D.W. & SOCOLOW, E.L. (1961) The source of lipid accumulation of L cells. *Journal of Biophysical and Biochemical Cytology*, **9**, 135.
- BRANDT, A.E. & LANDS, W.E.M. (1967) The effect of acylgroup composition on the rate of acyltransferase-catalysed synthesis of lecithin. *Biochimica et Biophysica Acta*, **144**, 605.
- BRODY, I. (1962) The ultrastructure of the epidermis in psoriasis vulgaris as revealed by electron microscopy. 3. Stratum intermedium in parakeratosis without keratohyalin. *Journal of Ultrastructure Research*, **6**, 341.
- COOPER, M.F., McGRATH, H. & SHUSTER, S. (1976) Epidermal lipid metabolism in psoriasis and lichen simplex. *British Journal of Dermatology*, **94**, 369.
- FOLCH, J., LEES, M. & SLOANE-STANLEY, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497.
- GRAY, G.M. (1967) Chromatography of lipids. I. An improved chromatographic procedure for the quantitative isolation of the neutral ceramide-containing glycolipids from mammalian tissues. *Biochimica et Biophysica Acta*, **144**, 511.
- GRAY, G.M. & YARDLEY, H.J. (1975) Lipid compositions of cells isolated from pig, human and rat epidermis. *Journal of Lipid Research*, **16**, 434.
- LANDS, W.E.M. & MERKL, I. (1963) Metabolism of glycerolipids. III. Reactivity of various acyl esters of coenzyme A with α' -acylglycerophosphorylcholine and positional specificities in lecithin synthesis. *Journal of Biological Chemistry*, **238**, 898.

Glucose tolerance in eczema

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SUMMARY

The glucose tolerance of thirty-nine patients with eczema was studied by the intravenous glucose tolerance test. The glucose disappearance rate for the eczema group was 1.69 ± 0.13 mg % per min and that for the controls was 2.92 ± 0.12 , indicating impairment of glucose tolerance in eczema patients.

Ten years ago the rate of glucose utilization in normal Egyptians was assessed by the intravenous glucose tolerance test (IVGTT). It was accidentally observed that a small group of subjects who were suffering from eczema but were otherwise 'normal' showed abnormalities in their glucose tolerance.

The aim of the present investigation is to verify this observation and to provide information about glucose tolerance in a larger group of patients with eczema.

PATIENTS AND METHODS

Thirty-nine patients suffering from eczema were studied. They consisted of 28 males and 11 females varying in age from 20 to 50 years (average 42 years). All were in-patients in the Dermatology Department of Ein Shams University Hospitals. Nine cases presented with acute eczema, fourteen patients with subacute eczema and sixteen cases with chronic eczema. The lesions were localized in ten cases and were disseminated in twenty-nine cases. Eight cases could be classified as allergic contact dermatitis, six as stasis dermatitis, two as photosensitive eczema and the remaining twenty-three cases could not easily be classified as they did not present a specific clinical picture conforming with any of the classical clinical forms. Subjects presenting with frank atopic dermatitis were not included.

Patients who had secondary infection or those who had recently been treated with systemic or topical corticosteroids were excluded.

The control group consisted of seventy-five normal Egyptians matched in terms of age, sex and body weight with the group of patients.

The intravenous glucose tolerance test was carried out under standard conditions. The test was performed in the morning after an overnight fast. The subject was at rest during the whole test. After drawing a fasting blood sample, a glucose load of 0.5 g/kg body weight was injected I.V. as a 50% solution.

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solution. Venous blood samples were collected at 10, 20, 40 and 60 min after the end of injection. Blood glucose was determined by autoanalyser employing the potassium ferricyanide-ferrocyanide oxidation-reduction reaction method. The blood glucose values were plotted against time in minutes on semilogarithmic paper. Glucose disappearance rate (K) in mg % per minute was calculated using the formula:

$$K = \frac{0.693}{m_{\frac{1}{2}}}$$

where $m_{\frac{1}{2}}$ equals the half life of glucose after injection calculated from the graph. Determinations were made of the mean values of the fasting blood glucose level, 60 min blood glucose level, 60 min blood glucose excess, which is the difference between the 60 min and the fasting levels, as well as the glucose disappearance rate (K). The standard deviation (σ), and the standard error of the mean (s.e.m.) were calculated. The results of the eczema group were compared to those of the control group using the Student's *t*-test and the probability *P* for each *t*.

RESULTS

The fasting blood glucose level of the eczema patients was 82.4 ± 2 (mean $\pm$ s.e.m.) and that of the control group 79.6 ± 1.4 mg %. The difference was not statistically significant ($P > 0.1$). Three patients (7.7%) had their fasting levels above the normal range (mean $+ 2\sigma$).

The mean 60 min blood glucose levels in patients and controls were 94.3 ± 6.3 and 74.7 ± 2.1 respectively; the difference was statistically significant ($P < 0.005$). The 60 min blood glucose level in ten patients (25.6%) was above the normal range. The mean 60 min blood glucose excess of patients was $+12 \pm 6.1$ in contrast to that of controls which was -5.9 ± 2.09 with a significant difference ($P < 0.05$). Seventeen patients (43.6%) had the 60 min blood glucose level above their fasting level.

The mean glucose disappearance rate was significantly lower in the group of patients than in the control group ($P < 0.001$), being 1.69 ± 0.13 and 2.92 ± 0.12 respectively. This is shown by the gradient of the slope of the glucose disappearance curve which is less steep in the group of patients (Fig. 1). Ten cases (25.6%) had their K value below the lowest limit of normal, which was set at 0.92 (Hamza, 1966).

TABLE 1. Results of IVGTT in eczema and control groups

Blood glucose	Eczema group			Control group			<i>P</i>
	Mean	s.d.	s.e.m.	Mean	s.d.	s.e.m.	
Fasting	82.4	12.3	2.0	79.6	12.3	1.4	> 0.1
10 min	199.7	86.3	10.6	223.7	46.7	5.4	< 0.05
20 min	166.3	55.7	8.9	167.9	41.8	4.9	> 0.49
40 min	120.4	45.2	7.2	100.6	34.1	4.1	< 0.02
60 min	94.3	39.1	6.3	74.7	18.2	2.1	< 0.005

DISCUSSION

Eczema is a distinctive pattern of inflammatory response of the skin induced by a wide variety of

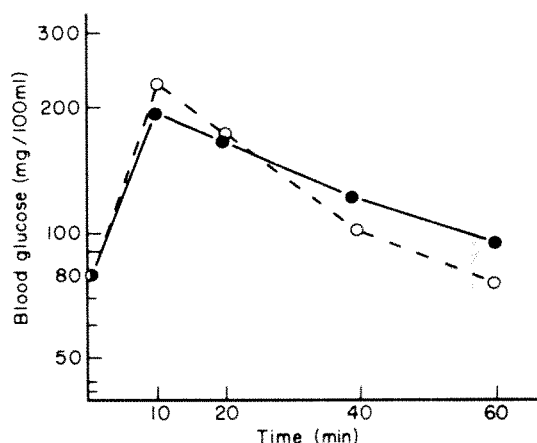


FIGURE 1. Mean glucose disappearance curve in control and eczema groups. (---) Normal; (—) eczema.

external and internal factors acting singly or in combination (Rook & Wilkinson, 1972). No generally accepted classification of eczema exists and many cases defy assignment to any definite type (Lever & Lever, 1975). Eczema, however, presents a characteristic and distinctive clinical as well as histopathological picture. In this context we consider the various types of eczema collectively as a type of reaction despite the various possible aetiological factors which may be implicated.

In the present study it was found that the group of eczema patients did not show obvious abnormalities in the basal state, that is the fasting blood glucose, yet they demonstrated reduced rates of glucose disappearance (K) and a significant increase in the 60 min blood glucose level and the 60 min blood glucose excess. The degree of impairment of glucose disappearance did not correlate with the extent, duration or type of the eczema reaction.

Some skin diseases have been reported to be associated with a greater than normal incidence of diabetes, such as vitiligo (Dawber, 1968), Kaposi's sarcoma (Fischer & Cohen, 1951), generalized papular forms of granuloma annulare (Romaine *et al.*, 1968), psoriasis (Reeds *et al.*, 1964) and porphyria cutanea tarda (Burnham & Fosnaugh, 1961). Recently it was shown that 42% of patients with active lichen planus and a negative family history for diabetes had unequivocally abnormal glucose tolerance (Lowe *et al.*, 1967). Similar findings were also reported by Powell *et al.* (1974). Jelineck (1970) suggested that the inheritance of diabetes is multifactorial and that it is possible that diabetes represents a phenotype common to several genotypes. The presence of a higher than normal incidence of chemical diabetes among patients suffering from eczema may be explained by assuming a common genetic link between the liability of the skin to react to various external factors by eczematization and the liability to the development of chemical diabetes. Further investigations are suggested to study this phenomenon more closely.

REFERENCES

- BURNHAM, T.K. & FOSNAUGH, R.P. (1961) Porphyria, diabetes and their relationship. *Archives of Dermatology*, **83**, 717.
- DAWBER, R.P.R. (1968) Vitiligo in mature onset diabetes mellitus. *British Journal of Dermatology*, **80**, 275.
- FISCHER, J.W. & COHEN, D.M. (1951) Simultaneous occurrence of Kaposi's sarcoma, leukemia and diabetes mellitus. *American Journal of Clinical Pathology*, **21**, 586.
- HAMZA, S.H. (1966) Studies on glucose tolerance tests. M.D. Thesis, Ein Shams University, Cairo, Egypt.

- JELINECK, J.E. (1970) The skin in diabetes mellitus. Cutaneous manifestations, complications and associations. In: *The Year Book of Dermatology* (ed. by A.W.Kopf and R.Andrade), p. 9. Year Book Medical Publishers, Chicago.
- LEVER, W.F. & LEVER, G-S (1975) *Histopathology of the Skin*, 5th edn, p. 96. Lippincott, Philadelphia.
- LOWE, N.J., CUDWORTH, A.G., CLOUGH, S.A. & BULLEN, M.F. (1967) Carbohydrate metabolism in lichen planus. *British Journal of Dermatology*, **95**, 9.
- POWELL, S.M., ELLIS, J.P., RYAN, T.J. & VICKERS, H.R. (1974) Glucose tolerance in lichen planus. *British Journal of Dermatology*, **91**, 73.
- REEDS, R.E., FUSARO, R.M. & FISHER, I. (1964) Psoriasis vulgaris. I. Clinical survey of association with diabetes mellitus. *Archives of Dermatology*, **89**, 205.
- ROMAINE, R., RUDNER, E.J. & ALTMAN, J. (1968) Papular granuloma annulare and diabetes mellitus. *Archives of Dermatology*, **98**, 152.
- ROOK, A.J. & WILKINSON, D.S. (1972) In: *Text Book of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J. Ebling), 2nd edn, p. 256. Blackwell Scientific Publications, Oxford.

Induction of UV light tolerance by PUVA in patients with polymorphous light eruption

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SUMMARY

A tan induced by 8-methoxypsoralen-long wave ultraviolet light has proved an effective photoprotective sunscreen in 5 patients with long wave ultraviolet light-induced polymorphic light eruption.

The most effective protection of skin against the damaging effects of ultraviolet light (UV) is provided by melanin pigmentation which effectively blocks the passage of damaging photons to the dermis (Pathak & Fitzpatrick, 1974); theoretically, one should, therefore, be able to employ the long-lasting tan induced by oral photochemotherapy (PUVA) as a photoprotective screen in photosensitivity disorders. This report deals with the effect of PUVA-induced tanning in patients with polymorphous light eruption (PLE) precipitated by long-wave UV light (UV-A, 320–400 nm).

PATIENTS

Five patients of skin type II and III (Melski *et al.*, 1977) with chronic PLE of at least 3 years' duration were selected for this study. The history of all patients indicated that the action spectrum of the disease was in the UV-A range (flares after exposure to the sun behind window glass, flares after indirect sun exposure in the shade, no protective effect of topical sun-screens, including those containing benzophenone and para-aminobenzoic acid (PABA)). The eruptions consisted of papular and papular-urticarial lesions, histologically characterized by dermal oedema and dense lymphocytic infiltrates, localized on light exposed areas of the body. The patients thus resembled some of the cases described by Frain-Bell *et al.* (1969). Phototests performed in three of these patients to verify that the action spectrum was in the UV-A range resulted in a positive test response to UV-A in all three (see below). The complete action spectrum was not determined.

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PHOTOTESTING FOR PLE

The phototesting procedure for PLE was based on the method of Epstein (1966), using an OSRAM high pressure 6000 W Xenon arc lamp, which emitted a continuous spectrum ranging from short-wave UV well into visible light (Fig. 1). A Mylar filter (Sams, 1967) was employed to block UV rays below 320 nm. After determination of the patient's individual minimal erythema dose (MED) with the unfiltered lamp, a 5×5 cm test area on the back was irradiated with the Mylar-filtered Xenon arc employing 10 times the MED. UV-A doses thus delivered ranged from 14.4 to 28.8 J/cm² of UV-A for each irradiation, as measured with a cosine corrected UV-A photometer (Ultraviolet Products, San Gabriel, Ca. USA). Irradiations employing identical doses were given to the same test sites daily for 3–5 consecutive days. The reproduction of the clinical lesions, typical for PLE, was considered a positive test response.

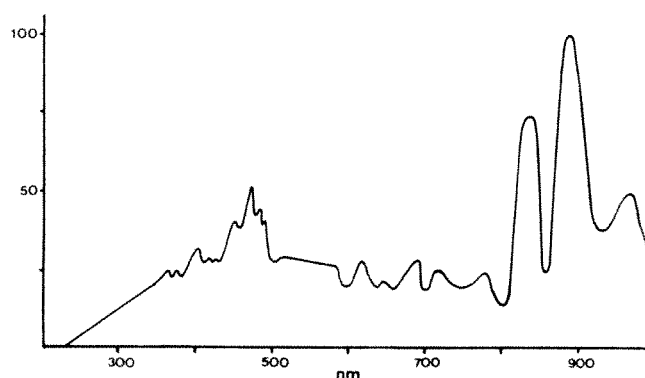


FIGURE 1. Emission curve of Xenon light source employed in UV-A testing (Xenon XBF 6000 W lamp (Osram)).

PUVA TREATMENT

PUVA treatment with oral 8-methoxypsoralen (8-MOP)* was initiated in May and June in order to provide a maximum tan during the summer months. Phototesting for PUVA treatment and PUVA dosimetry were performed according to established method (Wolff *et al.*, 1977) employing high intensity UV-A irradiation units (PUVA 4000, Waldmann, Schwenningen, FRG), and consisted of 4 PUVA exposures per week (initial doses of UV-A energy ranging from 1.0 to 3.0 J/cm²) until pronounced tanning had occurred; treatments were then stopped. The total number of exposures ranged from 6 to 10, the total UV-A energy given was 20.3 ± 14.3 J/cm² (mean $\pm$ s.d.).

RESULTS OF PUVA-INDUCED TANNING

PUVA treatment was well tolerated by all but one patient who responded with a single PLE flare during photochemotherapy; the eruption subsided after about 4 days despite continuation of PUVA.

After PUVA treatment patients were permitted to expose themselves to sunlight under conditions which had previously induced severe eruptions. Three patients remained completely free from PLE eruptions during the summer, although they spent their holidays in areas with intense solar radiation. Two had slight recurrences after extensive sun bathing at the Mediterranean Sea.

* Oxsoralen, Gerot Pharmaceuticals, Vienna.

The patients did not receive further PUVA treatment that year and returned during springtime of the next year with the signs and symptoms of new PLE eruptions. The patients were treated with PUVA again, the photoprotective effect during the following summer being similar to that observed after the first PUVA course.

COMMENT

The prevention of photodermatoses with an action spectrum in the long UV and visible light is usually a problem as most sunscreens do not provide sufficient protection against these wavelengths and opaque sunscreens, which do, are unpopular for cosmetic reasons. Imbrie *et al.* (1959) have shown that the tan induced by 8-MOP and solar radiation protects against UV-B and thus sunburn reactions. The present study demonstrates that PUVA-induced tanning provides reasonable photoprotection for UV-A-mediated PLE and that this protective effect may last 6–8 weeks after the last PUVA exposure. Surprisingly, PLE flares during PUVA exposures were observed in only one patient but this was probably due to the fact that the UV-A energy doses employed in the individual PUVA-‘treatments’ were less than the amount of UV-A required to elicit PLE eruptions when no phototoxic agent was employed. (In the UV-A testing procedure 14.4–28.8 J/cm² per session were necessary to induce PLE lesions, whereas only 1–3 J/cm² of UV-A were employed in the initial PUVA exposures.)

The accelerated and potentiated tanning response to PUVA requires less UV-A energy than comparable tanning by solar radiation. Since such a tan appears to be a highly effective natural ‘sunscreen’ in UV-A-mediated PLE it may find wider application as a photoprotective measure than just for this condition alone. Patients in whom lower doses of UV-A induce clinical flares may not benefit from this approach which, however, should prove even more effective in photodermatoses mediated by UV-B.

ACKNOWLEDGMENTS

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REFERENCES

- EPSTEIN, J.H. (1966) Polymorphous light eruption. *Annals of Allergy*, **24**, 397.
- FRAIN-BELL, W., MACKENZIE, L.A. & WITHAM, E. (1969) Chronic polymorphous light eruption. *British Journal of Dermatology*, **81**, 885.
- IMBRIE, J.D., DANIELS, F., JR, BERGERON, L., HOPKINS, C.E. & FITZPATRICK, T.B. (1959) Increased erythema threshold six weeks after a single exposure to sunlight plus oral methoxsalen. *Journal of Investigative Dermatology*, **32**, 331.
- MELSKI, J.W., TANENBAUM, L., PARRISH, J.A., FITZPATRICK, T.B., BLEICH, H.L. and 28 participating investigators (1977) Oral methoxsalen photochemotherapy for the treatment of psoriasis: a cooperative clinical trial. *Journal of Investigative Dermatology*, **68**, 328.
- PATHAK, M.A. & FITZPATRICK, T.B. (1974) The role of natural photoprotective agents in human skin. In: *Sunlight and Man* (Ed. by M.A. Pathak, L.C. Harber, M. Seiji and A. Kukita), p. 725. University of Tokyo Press, Tokyo.
- SAMS, W.M., JR (1967) The effect of ultraviolet light on a transparent plastic. *Archives of Dermatology*, **95**, 225.
- WOLFF, K., GSCHNAIT, F., HÖNIGSMANN, H., KONRAD, K., PARRISH, J.A. & FITZPATRICK, T.B. (1977) Phototesting and dosimetry for photochemotherapy. *British Journal of Dermatology*, **96**, 1.

Oral methoxsalen photochemotherapy of recalcitrant dermatoses of the palms and soles

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SUMMARY

PUVA therapy successfully cleared various dermatoses mainly confined to the palms and soles in 18 of 20 patients treated. The conditions treated were: plaque-type psoriasis, pustular psoriasis, endogenous eczema and persistent palmoplantar pustulosis. Seventeen patients were treated in a controlled study of PUVA therapy versus no treatment at all and in 16 of these patients the disease was cleared in the PUVA-treated areas while the untreated areas remained unchanged or deteriorated. Twelve of the 18 patients were maintained in a clear state by continued maintenance PUVA treatment over 6–31 months while 3 patients had a spontaneous remission and are free of disease off all treatment.

Palmar and plantar dermatoses are a common presentation in dermatology practice. Most cases are in the diagnostic categories of psoriasis or eczema. In addition, some patients are diagnosed as persistent palmoplantar pustulosis and although the aetiology of this condition is obscure, at least some cases are probably localized forms of pustular psoriasis. These conditions have a feature in common, namely, lack of an effective treatment. Topical corticosteroids, with or without occlusion, are most commonly used but in most cases produce little more than palliation. The response to tar and dithranol is similarly poor. The disability due to these diseases, when the palms and soles are involved, is out of all proportion to the surface area involved and therefore these dermatoses represent a significant therapeutic problem.

Oral psoralen photochemotherapy (PUVA) has been reported to be a useful treatment for both psoriasis (Parrish *et al.*, 1974; Wolff *et al.*, 1976; Melski *et al.*, 1977) and atopic eczema (Morison *et al.*, 1978) involving the general body surface. A brief uncontrolled report has been published on the successful use of PUVA in the treatment of four cases of palmoplantar pustulosis (Mizuno *et al.*, 1976) and anecdotal reports have been presented at meetings. Spontaneous remissions, although uncommon, do occur in this condition and the present study was therefore designed to evaluate, in a

controlled manner, the effectiveness of this therapeutic modality in the treatment of a variety of palmar and plantar dermatoses.

PATIENTS AND METHODS

Twenty patients with severe involvement of the palms and/or soles were treated. The age range of the group was 16–79 years, with a mean of 46 years. The diagnoses and extent of involvement are detailed in Table 1. Of the patients with endogenous eczema, two had subacute eczema with vesiculation and erythema while the other three patients had chronic hyperkeratotic eczema. All patients had bilateral disease and the duration of their disease ranged from 6 months to 30 years with a mean of 10 years. All patients had been treated previously with topical corticosteroids both with and without occlusion and two patients with psoriasis had received courses of methotrexate.

TABLE 1. Diagnosis and extent of involvement in the twenty patients entered into the study. Eight of the patients with disease on the palms also had involvement of the dorsa of the hands

<i>Diagnosis</i>	
Pustular psoriasis	7
Plaque-form psoriasis	5
Palmoplantar pustulosis	3
Endogenous eczema	5
<i>Extent of involvement</i>	
Palms and soles	14
Palms only	2
Soles only	4
Nails	10
Psoriatic arthritis	4

Design of the study

To evaluate critically the effect of the treatment, in 17 of the 20 patients PUVA was initially used on one area only and no treatment, apart from lubricants, was given for the other areas which served as a control. The right palm was treated first in 8 cases, the right sole was treated first in 4 cases and in 5 patients with involvement of both the palms and soles, both palms were treated while the soles served as control untreated areas. Control areas were shielded with two layers of cotton sheeting during irradiation. When the area treated initially had cleared completely and showed no evidence of disease, all involved sites were then treated with PUVA. The study was uncontrolled in 3 patients who received PUVA treatment to all involved areas from the start.

Other medications

Patients were permitted unrestricted use of emollients on both treated and control sites. All other dermatological medications, including topical corticosteroids, were ceased at the time of commencement of the study.

PUVA therapy

Oral 8-methoxypsoralen (0.6 mg/kg body weight) was ingested 2 h before exposure to UVA radiation.

The initial dose of UVA (range 2.5–4.5 J/cm²) was determined by the patient's sunburn and suntan history and this dose was increased by 0.5–1.0 J/cm² each treatment provided no significant erythema was present. Treatments were given three times a week during the clearance phase with a minimum of 48 h between treatments. Once a patient was completely free of disease a maintenance schedule was commenced of 4 treatments at weekly intervals followed by 4 treatments at fortnightly intervals and then monthly treatments. A recurrence of disease was managed by increasing the frequency of treatment with or without an increase of the UVA dose. Local irradiation was provided by a wall-mounted bank of PUVA fluorescent bulbs (Sylvania), the characteristics of which have been described (Levin & Parrish, 1974). The irradiance of this unit varied (7–12 mW/cm²) depending on the age of the bulbs and intensity was uniform ($\pm 10\%$) up to 15 cm away. Using this unit it was only possible to irradiate the palms, soles and dorsa of the hands. Anatomical considerations prevented effective irradiation of the dorsa of the feet.

Evaluation and laboratory investigations

Physician evaluations were made at 1–2 week intervals in the clearance phase of therapy and at 1–2 month intervals during maintenance. Before starting treatment all patients had a complete blood count, urinalysis, and estimation of serum bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase, lactic dehydrogenase, blood urea nitrogen, creatinine and antinuclear factor. These studies were repeated at 6-month intervals. In addition, all patients had a complete ophthalmological examination which was repeated yearly.

RESULTS

Eighteen of the 20 patients were completely cleared of their disease at all treated sites. Of the 17 patients who formed the controlled study, 16 were cleared of all disease at treated sites. During the initial phase of therapy when PUVA treatment was limited to one area, the control untreated sites in these 16 patients remained unchanged or deteriorated. Therefore at the end of this phase of the study it was possible to make a clear comparison between the areas treated with PUVA, which were free of disease, and the untreated control sites which were unchanged or worse. An example of the results achieved in this controlled phase of the study is illustrated in Fig. 1. At that time PUVA



FIGURE 1. A 22-year-old female patient with bilateral psoriasis of the palms required 22 PUVA exposures to clear the disease from the right palm. The left palm was shielded during this initial controlled phase of the study and remained unchanged.

treatment of the control sites was commenced and in the 16 patients in whom treatment was originally successful, the control sites were also cleared of disease. The results for treatment of all sites are summarized in Table 2. It can be seen that the palms and dorsa of the hands tended to clear quicker with fewer treatments and a lower final UVA dose as compared to the soles. However, the differences were not marked. When the results obtained in the four disease categories were compared there were no apparent differences between the diseases in terms of numbers of treatments or final UVA dose required to clear the disease.

TABLE 2. Treatment response in the 18 patients cleared of their disease

	% Patients (and no.)	No. days to clear	No. treatments to clear	Final clearance	
				dose UVA (J/cm ²) Mean	Range
Palms	94 (15/16)	53	16	12	6-28
Dorsa hands	100 (8/8)	40	14	13	6-28
Soles	89 (16/18)	62	22	17	9-28

Two patients, both with pustular psoriasis, were not cleared with PUVA therapy. One patient ceased treatment after twelve treatments due to the side effects of nausea and vomiting and at that time had shown improvement in the disease. The other patient was also improved so that <10% of the treated area was still involved by disease but he could not be completely cleared and an attempt to shift onto a less frequent maintenance schedule was unsuccessful. Three patients, two with eczema and one with psoriasis, had extension of their disease beyond the extremities while on treatment. The disease duration in these patients was 1-3 years and previous involvement had been limited to the hands and feet. PUVA successfully cleared these extensions of their disease and at follow-up 7-24 months later, maintenance therapy was required only for the hands and feet. The nails were involved in the disease process in the 7 patients with pustular psoriasis, 2 patients with psoriasis and in one patient with palmoplantar pustulosis. Of the 10 patients with nail involvement, six were cleared of disease at that site but the nails took many more treatments to clear than did the skin disease. In one patient with psoriatic arthritis, the joint symptoms settled at the same time as the skin disease. However, in three other patients the arthritis remained unchanged during the course of treatment.

Nausea, pruritus and erythema were observed in some patients as has been described in other studies of PUVA therapy (Melski *et al.*, 1977). However, the incidence of symptomatic erythema was surprisingly high with 20 episodes occurring in 9 patients. Erythemas occurred at all sites treated: palms, dorsa of hands and soles. One patient with psoriasis developed a Köbner response following an erythema and one patient stopped treatment during the maintenance phase, due to recurrent erythema. One cause of the high incidence of erythema was the presence of post-inflammatory depigmentation which appeared in four patients as their disease cleared. Depigmentation adjacent to areas of normally pigmented skin made correct dosimetry very difficult. Two patients developed scattered macules of hyperpigmentation on the palms but this became less prominent during maintenance therapy.

The 18 patients cleared of their disease have been followed for 6-31 months with a mean of 21 months follow-up. Twelve patients are clear of disease on regular maintenance treatments, the frequency being weekly in 5 patients and fortnightly in 7. Three patients have stopped treatment and are free of disease after 6-12 months. Three patients have stopped treatment and have had a recurrence

of their disease: one ceased due to recurrent erythema and two patients ceased treatment due to family and social reasons. Five patients, all with psoriasis, have had minor exacerbations of their disease during the stepwise reduction in the frequency of treatment on the maintenance schedule. These exacerbations were all readily controlled by an increased frequency of treatment. No new abnormalities have been detected in the laboratory investigations or eye examinations as compared to baseline studies.

DISCUSSION

This study has demonstrated that PUVA therapy is an effective clearance treatment in most cases of palmar and plantar dermatoses of the psoriasis and eczema group. Because of the variable course of these conditions a controlled study was necessary to define clearly the efficacy of this treatment. This has been achieved since in 16 of 17 patients who responded to the treatment, the untreated control sites remained the same or deteriorated during the period when PUVA treatment was applied to one area only. Subsequently the control sites were also cleared of disease. Topical corticosteroids, usually with occlusion, are the principal therapy presently available for treating eczema and psoriasis of the palms and soles but the response is usually very disappointing. This was evident in the present study since the patients had all been treated in the past with topical corticosteroids plus occlusion, often for many years, with poor responses. Therefore PUVA is an effective alternative treatment with a high response rate. The results of continued maintenance treatment were also very satisfactory since most patients could be maintained on an acceptable regimen of weekly or fortnightly treatments.

The treatment response in these patients resembles that observed in the whole-body PUVA therapy for psoriasis of the general body surface. The number of treatments required for clearance is in a comparable range and the final UVA dose was also similar (Melski *et al.*, 1977). Nail involvement was cleared in some patients and this took much longer to clear than did the skin involvement, while associated psoriatic arthritis did not appear to be affected in most patients; both these findings have been reported with whole-body treatment (Morison *et al.*, 1978). In the PUVA therapy of atopic eczema, patients must rigidly adhere to the maintenance schedule or risk developing a rapid flare-up of their disease. However, this rigid maintenance requirement was not observed in any of the patients in this study including those with eczema.

The side-effects of the treatment were not in most cases very marked. One patient ceased treatment due to nausea and vomiting and another patient chose to stop maintenance treatment because of recurrent symptomatic erythema. The number of such erythemas was high and the main reason for this was the variable pigmentary response seen in some patients. Clearing of the disease on the palms and soles can leave depigmented areas and this complicates dosimetry due to the wide disparity in the tolerance to therapy of adjacent pigmented and depigmented areas.

In this study 6 patients with disease on the soles also had involvement of the dorsa of the feet. However, it was not possible to treat the dorsa of the feet with the available radiation source. Since disease of the soles frequently extends onto the dorsa, specially designed radiators will be required for this treatment. The ideal radiation source should provide for simultaneous exposure of the whole of the hands and feet with a uniform, high-intensity irradiance. The irradiance should gradually taper up the forearms and legs so that there is no marked border to the pigmentation resulting from treatment. If this treatment becomes generally accepted such radiators will no doubt be produced.

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REFERENCES

- LEVIN, R.E. & PARRISH, J.A. (1975) Phototherapy of vitiligo. *Lighting Design and Application*, **5**, 35.
- MELSKI, J., TANENBAUM, L., PARRISH, J.A., FITZPATRICK, T.B. & BLEICH, H.L. (1977) Oral methoxsalen photochemotherapy for the treatment of psoriasis: a cooperative clinical trial. *Journal of Investigative Dermatology*, **68**, 328.
- MIZUNO, N., UEMATSU, S. & OHNO, M. (1976) Methoxsalen and irradiation treatment for pustulosis palmaris et plantaris. *Archives of Dermatology*, **112**, 883.
- MORISON, W.L., PARRISH, J.A. & FITZPATRICK, T.B. (1978) Oral psoralen photochemotherapy of atopic eczema. *British Journal of Dermatology*, **98**, 25.
- MORISON, W.L., PARRISH, J.A. & FITZPATRICK, T.B. (1978) Controlled study of PUVA and adjunctive topical therapy in the management of psoriasis. *British Journal of Dermatology*, **98**, 125.
- PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, L. & PATHAK, M.A. (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. *New England Journal of Medicine*, **291**, 1207.
- WOLFF, K., FITZPATRICK, T.B., PARRISH, J.A., GSCHNAIT, F., GILCHREST, B., HÖNIGSMANN, H., PATHAK, M.A. & TANENBAUM, L. (1976) Photochemotherapy for psoriasis with orally administered methoxsalen. *Archives of Dermatology*, **112**, 943.

Treatment of pustular psoriasis with clofazimine

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SUMMARY

Two cases of generalized pustular psoriasis (von Zumbusch) were successfully treated with clofazimine. No side effects were noted except deep red staining of the skin which faded afterwards.

Many systemic treatments have been used for the treatment of generalized pustular psoriasis especially in cases not undergoing spontaneous remission; for example, systemic corticosteroids, methotrexate, hydroxyurea and azathioprine (Baker, 1975). However, the well-known severe complications from these medications urge us to seek another treatment, especially in younger age patients.

This communication describes the successful use of clofazimine in two young male patients with generalized pustular psoriasis (von Zumbusch).

CASE REPORTS

Case 1

A 28-year-old male was admitted to the Institute of Dermatology in March, 1976 with a 1 month history of generalized skin pustulation. He had a 10-year history of scaly plaques on his scalp. The pustules were first noted on his forearm 1 month prior to admission and rapidly spread to the whole body. He also had high fever. On admission, generalized pustulation was seen all over his body, covering the entire skin surface except a few areas on the face, palms and soles. A geographical tongue was present. Nail dystrophy and onycholysis with subungual debris were also noted. The temperature was 39°C and a leukocytosis of 13,100 cells/mm³ was found. A skin biopsy from a pustule showed the histology of pustular psoriasis. Wet dressings with potassium permanganate for 5 weeks resulted in no improvement. New crops of pustules continued to appear nearly every 4 days. Dapsone 200 mg per day was given for 3 weeks, but methaemoglobinaemia developed and the lesions did not improve. Next trimethoprim-sulfamethoxazole was tried for 3 weeks without beneficial effect. Finally clofazimine 200 mg per day was given. Subsequently the pustulation rapidly disappeared in 4 days and the scales gradually diminished. The colour of the skin changed to a generalized bright erythema but there were no new pustules during the following 14 days. New crops of pustules were again seen when the dose of clofazimine was reduced to 100 mg per day. The dose was then increased again to 200 mg per day and the skin cleared in 8 days. Clofazimine was discontinued 3 weeks later without

any new pustulation developing. No side effects from the medication were noted except deep red staining of the skin due to deposition of the dye and which gradually faded after the drug was discontinued. During follow-up every 2 months for 1 year only mild scaling on the scalp was observed.

Case 2

A 20-year-old Thai male has had a history of three severe attacks of generalized pustulation accompanied by high fever and leukocytosis since 17 years of age. He also had ankylosing deformities of the finger joints of both hands. The histopathology from the skin biopsies was that of pustular psoriasis; the clinical impression was that of the von Zumbusch type. Recurrent pseudomonas infections in the middle ear seemed to be the precipitating cause. His symptoms and pustulation would persist if he was treated symptomatically without methotrexate. A single oral dose of 50 mg per week of methotrexate regularly controlled the eruption. Subsequently, he was maintained on a dose of 20 mg per week without skin pustulation for many months. Discontinuation of methotrexate would result in recurrence of a few crops of pustules. In December 1976, while he was receiving the maintenance dose, new crops of pustules recurred on the scalp, face, trunk and extremities accompanied by high fever and leukocytosis. A geographical tongue was also present. He was admitted and methotrexate was discontinued. Clofazimine 200 mg daily was given orally. The crops of pustules were reduced in numbers within 5 days and there were no new lesions within 21 days. The skin changed to a mild exfoliative dermatitis and improved with topical cold cream. He was discharged with clofazimine 200 mg daily for another 2 months after which medication was discontinued. No side effects were noted from the clofazimine except a deep red staining of the skin which gradually faded. He has been followed up for 5 months without recurrent skin pustulation.

DISCUSSION

Clofazimine, a phenazine imino-quinone derivative, has been used for the treatment of leprosy, discoid lupus erythematosus (Mackey & Barnes, 1974), pyoderma gangrenosum (Michaëlsson *et al.*, 1976), and palmo-plantar pustulosis (Molin, 1975). The mechanism of action in leprosy is possibly by bacteria-DNA binding activity (Morrison & Marley, 1976). In pyoderma gangrenosum and palmo-plantar pustulosis it possibly acts by enhancing phagocytic activity of the leukocytes (Michaëlsson *et al.*, 1976; Molin, 1975). The mechanism of action in psoriasis is not known since no studies on DNA binding activity or on the effect on the migration of leukocytes have been done in this disease.

In order to evaluate the efficacy of new drugs, time should be allowed for spontaneous remission in any case of pustular psoriasis. Up till now the longest course of generalized pustular psoriasis observed at our Institute before spontaneous remission has been 52 days (Piamphongsant, 1977). In our first case, spontaneous remission was not observed even after 11 weeks. Once clofazimine was started, the pustules regressed in 4 days. It is possible that this could have been a spontaneous remission, but the fact that remission did not take place before clofazimine was instituted, favours the efficacy of the drug. Besides, when the dosage was reduced the pustules recurred and could be controlled again by reinstituting the higher dosage. The remission period was quite long. In our second pustular case, which was methotrexate dependent, clofazimine was substituted and discontinued without a recurrence of pustulation.

Although the course of pustular psoriasis is variable and unpredictable, its response to treatment in our cases favoured the efficacy of clofazimine in treating certain cases of pustular psoriasis. It is interesting, however, that clofazimine was also tried in 6 cases of discoid psoriasis without success (Mackey, 1976).

REFERENCES

- BAKER, H. (1975) Psoriasis: a review. II. *Dermatologica*, **150**, 136.
- MACKEY, J.P. & BARNES, J. (1974) Clofazimine in the treatment of discoid lupus erythematosus. *British Journal of Dermatology*, **93**, 91.
- MACKEY, J.P. (1976) Clofazimine in Dermatology. *International Journal of Dermatology*, **15**, 140.
- MICHAËLSSON, G., MOLIN, L., OHMAN, S., GIP, L., LINDSTROM, B., SHOGH, M. & TROLIN, I. (1976) Clofazimine, a new agent for the treatment of pyoderma gangrenosum. *Archives of Dermatology*, **112**, 344.
- MOLIN, L. (1975) Clofazimine—enhanced phagocytosis in pustulosis palmaris et plantaris. *Acta Dermatovenereologica*, **55**, 151.
- MORRISON, N.E. & MARLEY, G.M. (1976) The mode of action of clofazimine—DNA binding studies. *International Journal of Leprosy and other Mycobacterial Diseases*, **44**, 133.
- PIAMPHONGSANT, T. (1977) Generalized non-discoid pustular psoriasis: Its histodynamic. *Journal of the Medical Association of Thailand* (in press).

Assessment of the skin penetration properties of different carrier vehicles for topically applied cortisol

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SUMMARY

Four commercially available topical preparations of cortisol, Alphaderm, Calmurid HC, Efcortelan and Topisone, had trace quantities of tritiated cortisol added to them. The resultant radiolabelled creams were applied to the dorsum of the pig and the penetration of the steroid was assessed. Alphaderm was shown to deliver ~ 16.0 times more cortisol to the skin than Topisone, ~ 8.0 times more than Efcortelan and ~ 3.0 times more than Calmurid HC.

The methods available for the determination of percutaneous drug absorption *in vivo* include autoradiography, the 'disappearance technique' described by Hediger (1928), the 'direct uptake' methods of Parekh, Min & Goldberg (1970) and Polano & de Beukelaar (1968), and indirect measurements of plasma and urine drug concentrations such as those described by Feldmann & Maibach (1965) and Maibach (1976).

Measurement of the penetration profile of a drug within the histologically differentiated layers of the skin is a difficult technique without the use of radioisotopes and although autoradiography has been used for this purpose, difficulties arise with the steroids due to their solubility in histological solvents.

The purpose of this comparative study was to investigate the efficiency of percutaneous penetration of the cortisol free alcohol from different commercially available topical preparations.

Since all the preparations contained cortisol free alcohol the differences between them can only be attributed to the vehicles in which it is dissolved. In order to describe the total entities of steroid and base concisely the proprietary names for the products will be used throughout this paper.

In order to detect the presence of cortisol and quantify its level within the epidermis and dermis a trace quantity of tritium labelled cortisol was added to each preparation and allowed to equilibrate with the radioactively inert cortisol content. The added quantity of labelled cortisol (4.26×10^{-8} g/g

of preparation) was considered insignificant in terms of increased concentration of the pharmacologically active ingredient for practical purposes. Three of the topical preparations contained 1% cortisol and one contained 0.1% cortisol. Therefore, the ratio of added tracer material to that native to the preparation would be $1:23 \times 10^4$ and $1:23 \times 10^3$ respectively for the two levels of active ingredient.

The tritiated cortisol was labelled at carbon positions 1, 2, 6, and 7 in the A and B rings of the cyclopentanophenanthrene steroid structure. The biotransformation of the cortisol and its effect on stability of the tritium label is of importance in the interpretation of results. The metabolism of cortisol by slices of human skin from different anatomical sites was studied by Hsia & Lee (1966). They found that oxidation at C-11 and reduction at C-20 occurred in skin from differing anatomical sites, but reduction of the olefin and 3-oxo-group of cortisol was limited to the foreskin. The stability of the C-1, C-2 tritium label would depend on the A-ring conformation being retained and therefore these findings would indicate that loss of isotope at these positions is less likely to be encountered in studies on skin areas other than that of the foreskin. Any loss of A-ring conformation would tend towards exchange of tritium with tissue water and would have a short biological half life measurable in hours rather than days. Therefore, computation of cortisol levels would result in lower values than those actually present.

Porcine skin was chosen since there is evidence that some anatomical, histochemical and penetration features are shared with that of man (Montagna & Yun, 1964; Forbes, 1969; Winter & Wilson, 1976; Bartek & LaBudde, 1975). Bartek, LaBudde & Maibach (1972) showed that of rat, rabbit and pig the permeability of weanling swine is close to that of human skin.

The Association of the British Pharmaceutical Industry guidelines for preclinical and clinical testing of new medicinal products (1977) advocates the use of piglets because their skin is similar in many ways to that of man.

However, as the skin used in these studies was that of young white piglets, and because of the anatomical differences demonstrated by Hsia & Lee (1966), quantification of the measured radio-activity in terms of cortisol concentration could be affected by some unknown proportion of exchanged isotope at positions C-1 and C-2.

MATERIALS AND METHODS

Animal protocol

Five-week-old piglets (four) were acclimatized to handling for 1 week prior to application of the labelled preparations. The dorsal skin was washed and shaved at the beginning of the acclimatization period and marked out into five zones on each side of the mid-line, using 35 mm film mounts as templates, producing an aperture 23.5×34.5 mm (8.1 square centimetres). The zones were coded for the various cream types and each piglet received two creams, one to each side.

A fresh film mount was applied over the appropriate zone and 0.5 g of the appropriate cream was discharged onto the skin from a pre-loaded, tuberculin syringe without needle. The cream was rubbed into the masked skin area for a period of 2 min by means of a finger protected by a polythene glove. The film mount template was then removed and discarded. After application of the creams the back of the animal was covered with wide mesh, fused-junction, nylon gauze strapped at the outer edges with adhesive tape. The creams were applied twice daily with an 8 h interval. The start of application was staggered in order that at the time of death, areas of application had received dosage periods of 102, 78 and 54 h respectively, the last application being 6 h prior to death. At death blank areas to which no cream had been applied were taken for background skin radio-activity and comparative normal histology.

One sample was accidentally omitted from the Efcortelan 54 h area on one pig, and the percentage

dose corrected for this, by the subtraction of the amount of radioactivity calculated to be present in the missed dose. It is accepted that repeated applications do not produce an additive amount of penetration (Maibach, 1976).

Just prior to death the skin areas were wiped over with tissue and then washed with soap and water. The animal was anaesthetized with veterinary nembutal and the skin zones were incised by means of a scalpel using a 35 mm film mount as a guide. The areas were then excised individually to the depth of the subcutaneous fat, placed in coded containers and rapid frozen at $\sim -60^{\circ}\text{C}$. The animal was then killed. The tissue was then maintained at $\sim -30^{\circ}\text{C}$ until sectioned by freezing microtome.

Microtome sectioning

An 11 mm diameter cork borer was used to punch discs of tissue from the excised skin and provide a constant area for section cutting. The discs were punched from the subcutaneous side through to the skin surface to avoid carriage of radioactivity from the surface as a contaminant of deep layers of tissue.

A disc of 25% aqueous gelatin gel was cut by means of a 14 mm cork borer, frozen to the microtome stage trimmed parallel by the mounted blade. This provided a platform to which the tissue was frozen for sectioning. This platform ensured that the skin surface was parallel to the blade arc and provided a continuum for the tissue that would not damage the blade as the last section was cut. When the whole tissue block was frozen throughout, coarse sections were cut until the subcutaneous fat was observed to be removed. 10 μm sections were then cut through the dermis, moving through the epidermis towards the stratum corneum. As each section was cut it was transferred to tissue solubilizer in a counting vial for liquid scintillation counting in Dioxane-Toluene-PPO-POPOP phosphor solution. Channel ratio external standardization was used and counting continued until 1% accuracy was reached. Using this technique the tissue depth error incurred as the microtome blade approached the platform block is 0–10 μm .

Portions of the excised tissue were placed in 5.0% buffered formol Ringer for wax embedding. Sections from these preparations were cut and stained by haematoxylin and eosin.

Sudan lipid stain was applied to sections cut from fixed tissue on a cryostatic microtome.

Data processing

The data was processed by means of a Sumlock-Compucorp 445, programmed to correct for counting efficiency, specific activity of each topical preparation and sectioned skin area, to provide data in the form of $\mu\text{Ci}/\text{cm}^2$ of skin per 10 μm section. The mean of two sections from two skin areas from one application zone was used for histogram and table presentation. In order to encompass the wide range of radioactivity encountered, for comparative plotting in histogram form it was found most convenient to combine the values for $3 \times 10 \mu\text{m}$ providing 30 μm steps. The Y axis was then plotted as the root of $\mu\text{Ci} \times 10^{-6}/\text{cm}^2$.

The topical preparations

Four commercially available preparations were used for comparison—these were:

Alphaderm—1.0% Cortisol + 10% urea in a powder in cream base (Eaton Laboratories).

Topisone—0.1% Cortisol in a propylene glycol/water base (Syntex Pharmaceuticals).

Efcortelan—1.0% Cortisol in a water miscible cream (Glaxo Laboratories).

Calmurid HC—1.0% Cortisol + 10% urea in a stabilising emulsified base (Pharmacia).

(1,2,6,7,3H)-pregn-4-en-3-one-11 β , 17 α , 21 triol (Radio Chemical Centre, Amersham) 85 Ci/mmol, was diluted in 95% ethanol to a concentration of 2 mCi/ml. 0.1 ml (200 μCi) was evaporated onto the

surface of a Petri dish. 20 g of the particular topical preparation was weighed into the dish and mixed by means of a bevel ended P.T.F.E. spatula using a squeegee action over the surface of the Petri dish. The preparation was then transferred to a borosilicate glass vial and stored at $\sim 20^{\circ}\text{C}$ for 2 weeks prior to the determination of specific activity. Approximately 10 mg weighed to within 0.05 mg of topical preparation was dispersed into a water miscible scintillant phosphor solution for scintillation counting (Nuclear Chicago Isocap 300).

The topical preparations were loaded into 20 ml syringes and by means of a double ended luer adaptor, 1 ml tuberculin syringes were filled with 0.5 g samples of material, the volume of the sample being adjusted for the density of the particular preparation. The syringe nozzles were capped and the syringes coded for the various skin areas of application.

PREPARATION ASSAY

The means of ten determinations on random samples taken from throughout the final preparation of each cream are shown in Table 1. Included in Table 1 are the calculated specific activities of the cortisol content, assuming 95% confidence of label content of the cream and equilibration of the added radiolabelled cortisol. As can be seen from Table 1 there is a considerable variation in specific activity of the different final preparations. This variation is attributed to the differing capacity of the particular preparation in surface tension removal of the steroid from the Petri dish within the time spent in mixing action with the spatula, as well as lack of efficiency in covering the whole area of radiolabelled deposit with the squeegee action of the spatula blade. However, since all counting times were adjusted to give 1% statistical error, and all final tissue counts were corrected by the factor given in the last column of Table 1, the comparative tissue content is corrected for all topical

TABLE 1

Material	$\mu\text{Ci/g}$	Cortisol sp. activity ($\mu\text{Ci/mg}$)		Tissue count correction factor
		Calculated	Found	
Efcortelan	10.35	1.089	—	$\times 0.9662$
Calmurid HC	9.78	1.029	—	$\times 1.0225$
Alphaderm	6.77	0.713	0.690	$\times 1.4771$
Topisone	4.92	5.179	4.710	$\times 2.0325$

preparations to a level of 10 $\mu\text{Ci/g}$ of topical preparation.

Since the largest corrections were applied to Alphaderm and Topisone the cortisol content of both preparations was determined as being within 95% of label. The composition of both materials was multiphasic, so the specific activity of the cortisol in their major bulk phase was confirmed as being close to that calculated from the specific gravity of the total material on an assumed 95% confidence of label content. Assay by extraction and analysis by H.P.L.C. gave values of percentage label for Alphaderm and Topisone of 97% and 104% respectively.

Samples of the two materials were then centrifuged at approximately 7000 g for 1 h. Alphaderm separated into a solid particulate phase and an upper lipid phase and Topisone separated into a lower clear fluid phase and an upper lipid-like phase; incomplete break of an emulsion was present at the interface. Samples of the upper phase for Alphaderm and the lower phase for Topisone were taken

for H.P.L.C. analysis and liquid scintillation counting—the measured specific activities found are given in Table 1, and are within 10% of those calculated.

RESULTS

Figs 1–3 are histogram plots showing the magnitude of radioactivity at increasing depth from the stratum corneum for 54, 78, and 102 h periods of application. The data represent radioactivity found, corrected to an equal applied dose, i.e. 10 $\mu\text{Ci/g}$ cream for all topical preparations. It is apparent from Figs 1 and 2 that there is no significant difference between Alphaderm, Topisone, Calmurid HC and Efcortelan after 54 h application and after 78 h there is still little difference between Alphaderm, Topisone and Calmurid HC. However, there is an unaccountable lower level of radioactivity measured at 78 h for Efcortelan.

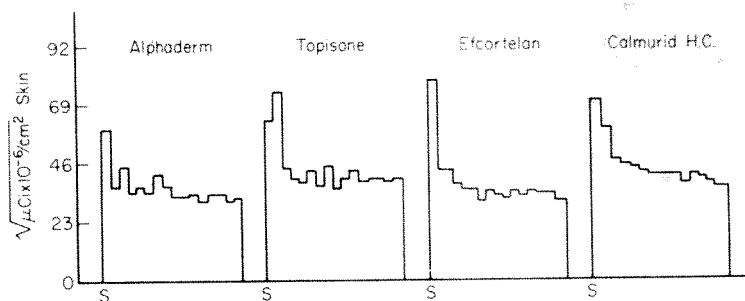


FIGURE 1. Radioactive profile in skin in 30 μm sections at 54 h. S, stratum corneum.

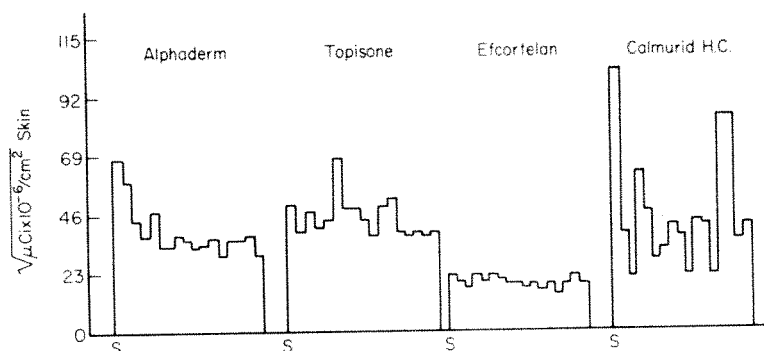


FIGURE 2. Radioactive profile in skin in 30 μm sections at 78 h. S, stratum corneum.

Examination of Fig. 3 shows a considerable increase in radioactivity at 102 h for Alphaderm in the zone from 240–330 μm from the skin surface. A similar profile at a lower level is seen for Topisone. There is no significant difference at 102 h for Efcortelan over those seen at 54 h (Fig. 1). The total radioactivity recovered from the application sites and expressed in terms of percentage of the total applied radioactivity is given in Table 2 for the three time periods. At 54 h Alphaderm and Efcortelan are almost equal in percentage of applied dose at 0.67% and 0.86%. Whereas Calmurid HC and Topisone show equal but higher values of ~0.96 and 0.99%. At 78 h there is no significant difference between Alphaderm, Topisone and Calmurid HC, but Efcortelan reflects the lower value seen in

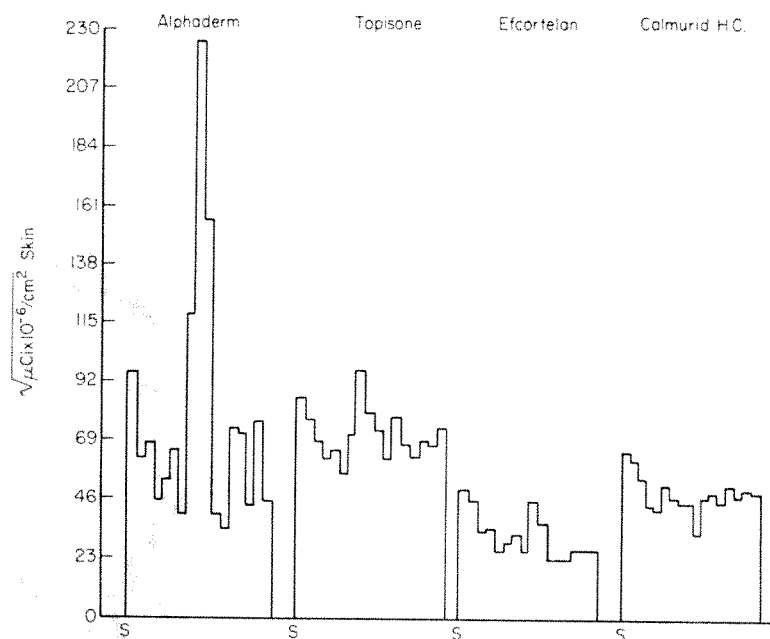


FIGURE 3. Radioactive profile in skin in 30 µm sections at 102 h. S, stratum corneum.

TABLE 2

Material	54 h		78 h		102 h	
	µCi Total	% Applied	µCi Total	% Applied	µCi Total	% Applied
Efcortelan	0.1725	0.8627	0.0477	0.1363	0.1482	0.3293
Calmurid HC	0.2397	0.9588	0.2608	0.7451	0.3288	0.7307
Alphaderm	0.1668	0.6672	0.2373	0.6780	1.0740	2.3867
Topisone	0.2478	0.99	0.2494	0.7126	0.7014	1.5587

Fig. 2. At 102 h there is a distinct rise in the value for Alphaderm with the remaining preparations in falling order of Topisone, Calmurid HC and Efcortelan.

The radioactivity can be calculated in terms of cortisol delivered from the total area of application by the use of the specific activity calculated on the basis of 95% confidence of stated cortisol content as given in Table 1, and expressed as percentage of total cortisol dose. Table 3 gives the data for 54, 78 and 102 h. From this table it is evident that at 54 h, Topisone and Calmurid HC have delivered the highest percentage of applied dose, but the lowest cortisol mass for Topisone is consistent with the fact that the preparation contains 10% of the concentration of cortisol relative to the other preparations. Alphaderm at ~0.6 is the lowest and Efcortelan is ~0.8% of applied dose. There is no significant change at 78 h apart from a fall in the percentage of total dose for Topisone. At 102 h Calmurid HC has maintained its percentage delivered at ~0.7-0.9, Topisone has risen to ~1.5% and Alphaderm to ~2.4% although the total mass delivered for Topisone is a reflection of its lower cortisol content, being half that of Efcortelan, both at 78 and 102 h.

TABLE 3. Area of application 8.1 cm²

Material	54 h		78 h		102 h	
	mg Cortisol from radioactivity	% Applied	mg Cortisol from radioactivity	% Applied	mg Cortisol from radioactivity	% Applied
Efcortelan	0.159	0.795	0.044	0.126	0.136	0.3022
Calmurid HC	0.233	0.932	0.254	0.726	0.320	0.711
Alphaderm	0.159	0.633	0.225	0.645	1.066	2.370
Topisone	0.024	0.942	0.024	0.675	0.066	1.476

DISCUSSION

The percutaneous penetration of steroids is dependent on skin factors and vehicle factors.

Feldmann & Maibach (1965) using urinary excretion of ¹⁴C-labelled cortisol showed that there was a prolonged absorption and excretion of drug by the topical route. It is assumed that the stratum corneum acts as a reservoir for the drug as well as a barrier to its penetration. However, stripping the stratum corneum does not remove the whole of the barrier to percutaneous absorption.

Elias & Friend (1975) have shown that the granular layer functions as an effective barrier to penetration from within the epidermis to the surface; it is likely that it will also act as a barrier to diffusion in the reverse direction. The keratinocytes, the skin appendages and the basement membrane also contribute to the total skin barrier (Feldmann & Maibach, 1965).

Specific lipid staining showed that for Efcortelan there was a large amount of lipid residing in the stratum corneum at 102 h (Fig. 4). This lipid depot was much less with Alphaderm (Fig. 5); however, Alphaderm produced a high lipid content in the deeper layers of the epidermis whereas Efcortelan did not.

Barry (1975) claims that diffusion through the horny layer is a passive process and is affected only by physical factors.

Poulsen *et al.* (1968) have shown that the percutaneous delivery of a drug is influenced by its concentration in the vehicle, its partition coefficient between the vehicle and the skin and its solubility in the vehicle.

Barry (1975) also points out that although the stratum corneum acts as a barrier the amount of material it can hold is small. Repeated application of steroid to the surface of the skin will result in the formation of a steady state reservoir. The level of this skin reservoir will be influenced by the rate of delivery of steroid to the skin, and its removal into the systemic circulation, and by the binding of the steroid and its metabolites to local sites.

This reservoir will be subject to the effects of the vehicle on the skin itself and the blood vessels within it.

Substances such as DMSO, DMF and DMA reversibly alter the barrier function of the skin (Barry, 1975; Sarkany & Gaylorde, 1976; Maibach & Feldmann, 1967).

Ayres (1977) has shown that the quantity of cream absorbed by human skin can be increased by adding and increasing the concentration of urea in the cream, indicating a barrier change as a response to urea. Feldmann & Maibach (1974) showed that the addition of 10% urea to a cream containing 1% cortisol acetate doubled the percutaneous absorption of the steroid. Hindson (1971) showed that the addition of urea to Betnovate significantly improved its clinical effectiveness.

The radioactivity measured in these studies was associated with the cyclopentanophenanthrene

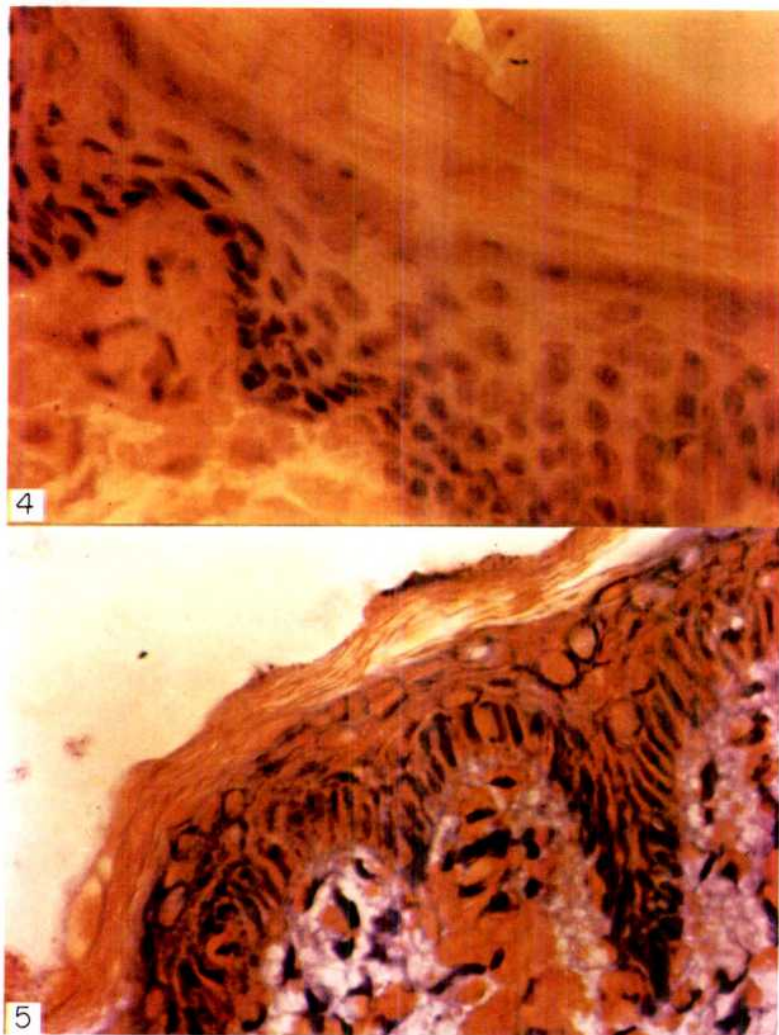


FIGURE 4. Section of pig skin treated with Efcortelan and stained with Sudan red (102 h). Note lipid in stratum corneum.

FIGURE 5. Section of pig skin treated with Alphaderm and stained with Sudan red (102 h). Note lipid in middle layers of epidermis.

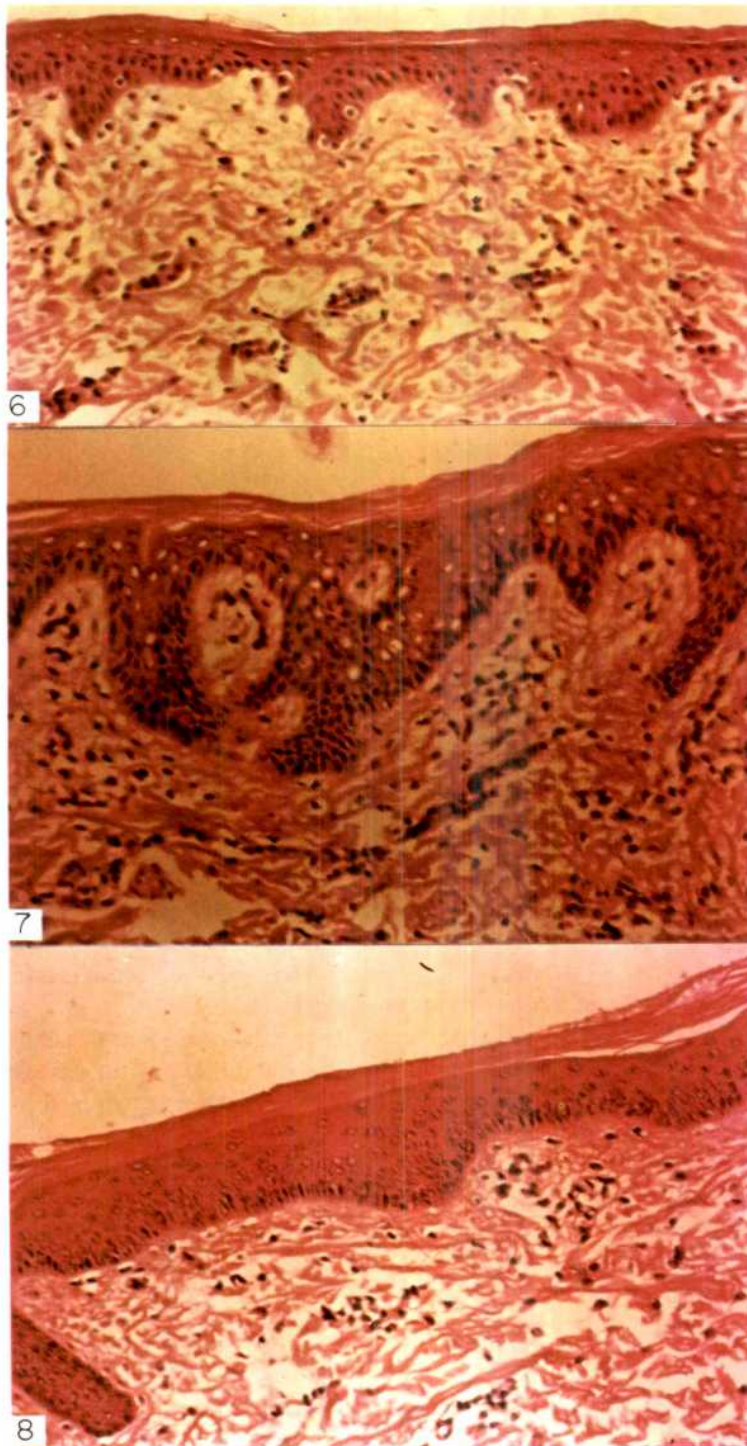


FIGURE 6. Section of pig skin treated with Topisone and stained with Haematoxylin and Eosin (102 h). Note relatively intact stratum corneum.

FIGURE 7. Section of pig skin treated with Alphaderm and stained with Haematoxylin and Eosin (102 h). Note separation of stratum corneum.

FIGURE 8. Section of pig skin treated with Efcortelan and stained with Haematoxylin and Eosin (102 h). Note relatively intact stratum corneum.

nucleus of the steroid structure and therefore it reflects the presence of either the primary steroid or its metabolites.

Table 4 shows the ratio of radioactivity at 102 h at a depth of 240–330 μm from the stratum corneum, the level at which peak radioactivity was encountered. This may be taken to indicate the penetrating potential of each preparation for its steroid moiety. Alphaderm has a penetration potential 25 times greater than that of Efcortelan, 16 times greater than that of Calmurid HC and four times greater than that of Topisone.

TABLE 4. Depot formation

Material	Ratio
Alphaderm	23.4573
Topisone	5.1807
Calmurid HC	1.5531
Efcortelan	1.0000

Ratio of radioactivity at 102 h between 240 and 330 μm depth from stratum corneum, Efcortelan value taken as 1.00.

Table 3 shows the total weight of cortisol delivered to the treated area of the skin, as calculated using 4 pigs. At 102 h Alphaderm had delivered ~ 16 times more than Topisone, ~ 8 times more than Efcortelan and ~ 3 times more than Calmurid HC. This strongly supports the view expressed above that urea alters the skin barrier function.

Whilst the histological techniques employed in this experiment cannot be used to demonstrate changes in the stratum corneum, histological examination of the pig skin in this study suggested that the stratum corneum remained relatively compact in the areas treated by Efcortelan (Figs 4 and 8) and Topisone, but that treated with Alphaderm (Figs 5 and 7) showed considerable separation of its layers. The finding is worthy of more specific investigation.

The results have shown that there is a marked difference in the amount of radioactivity delivered to the band of tissue lying at a depth of 240–330 μm from the surface, by the different creams. There is more lipid in the epidermis of the sections treated with Alphaderm than in that treated with the other creams. There is also more hydrocortisone in the skin areas treated with Alphaderm. The findings of Ayres (1977), Hindson (1971) and Feldmann & Maibach (1974) described above all indicate an increase in the efficiency of steroids in the presence of urea. This change in efficacy may well be produced because there is an improved lipid pathway through the skin in the presence of urea.

Maibach & Feldmann (1967) showed that simple occlusion could increase the penetration of cortisol tenfold. The effect of the delivery system in Alphaderm has been to equate the penetration of cortisol from the cream alone to that obtained by Maibach & Feldmann (1967) with occlusion.

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REFERENCES

- ASSOCIATION OF THE BRITISH PHARMACEUTICAL INDUSTRY (1977) *Guidelines for Preclinical and Clinical Testing of New Medicinal Products*. Part 1—Laboratory investigations.
- AYRES, P.J.W. (1977) Pharmaceutical developments in the production of delivery systems for treating ichthyotic conditions. In: *The Ichthyoses* (Ed. by R.Marks and P.J.Dyke) p. 167. Cardiff. M.T.P. Press.
- BARRY, B.W. (1975) Medicaments for topical application: Biopharmaceutics of dermatological preparations. *Pharmaceutical Journal*, **215**, 322.
- BARTEK, M.J. & LABUDDE, J.A. (1972) Percutaneous absorption. In: *Animal Models in Dermatology: Relevance to Human Dermatopharmacology and Dermatotoxicology*, (Ed. by H.Maibach), p. 103, Churchill-Livingstone, Edinburgh.
- BARTEK, M.J., LABUDDE, J.A. & MAIBACH, H.I. (1972) Skin permeability *in vivo*: comparison in rat, rabbit, pig and man. *Journal of Investigative Dermatology*, **58**, 114.
- ELIAS, P.M. & FRIEND, D.S. (1975) The permeability barrier in mammalian epidermis. *Journal of Cell Biology*, **65**, 180.
- FELDMANN, R.J. & MAIBACH, H.I. (1965) Penetration of ^{14}C Hydrocortisone through normal skin. *Archives of Dermatology*, **91**, 661.
- FELDMANN, R.J. & MAIBACH, H.I. (1974) Percutaneous penetration of hydrocortisone with urea. *Archives of Dermatology*, **109**, 58.
- FORBES, P.D. (1969) Vascular supply of the skin and hair in swine. In: *Advances in Biology of Skin* (Ed. by W.Montagna and R.L.Dobson), Vol. 9, p. 419. Pergamon Press, New York.
- HEDIGER, S. (1928) Experimentelle Untersuchungen über die Resorption der Kohlensäure durch die Haut. *Klinische Wochenschrift*, **7**, 1553.
- HINDSON, T.C. (1971) Urea in the topical treatment of atopic eczema. *Archives of Dermatology*, **104**, 284.
- HSIA, S.L. & LEE, H.Y. (1966) Metabolic transformations of cortisol-4- ^{14}C in human skin. *Biochemistry*, **5**, 1469.
- MAIBACH, H.I. (1976) *In vivo* percutaneous penetration of corticoids in man and unresolved problems in their efficacy. *Dermatologica*, **152**, Suppl. 1, 11.
- MAIBACH, H.I. & FELDMANN, R.J. (1967) The effect of DMSO on percutaneous penetration of hydrocortisone and testosterone in man. *Annals of the New York Academy of Sciences*, **141**, 423.
- MONTAGNA, W. & YUN, J.S. (1964) The skin of the domestic pig. *Journal of Investigative Dermatology*, **43**, 11.
- PAEKH, C., MIN, B.H. & GOLDBERG, L. (1970) Experimental studies of sodium pyridinethione. I. Percutaneous absorption in laboratory animals. *Food and Cosmetics Toxicology*, **8**, 147.
- POLANO, M.K. & DE BEUKELAAR, L. (1968) Penetration studies with ^{14}C labelled fluocinolone acetonide. In: *XIII Congressus Internationalis Dermatologiae Berlin*, p. 176. Springer-Verlag, Berlin.
- POULSEN, B.J., YOUNG, E., COQUILLA, V. & KATZ, M. (1968) Effect of topical vehicle composition on the *in vitro* release of fluocinolone acetonide and its acetate ester. *Journal of Pharmaceutical Science*, **57**, 928.
- SARKANY, I. & GAYLORDE, P.M. (1976) Effects of bases and accelerants on the anti-inflammatory activity of topical corticosteroids. *Dermatologica*, **152**, Suppl. 1, 81.
- VICKERS, C.F.H. (1966) Percutaneous absorption. In: *Modern trends in dermatology*, Vol. 3, p. 84. Butterworths, London.
- WINTER, G.D. & WILSON, L. (1976) Corticosteroid induced atrophy in the skin of the domestic pig. In: *Mechanisms of Topical Corticosteroid Activity* (Ed. by Lyn C.Wilson and R.Marks), p. 77. Churchill Livingstone Edinburgh.

Oral treatment of ichthyosis with an aromatic retinoid

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SUMMARY

An aromatic retinoid (Ro-10/9359) was used for oral treatment of five cases of ichthyosis (three lamellar, two X-linked).

Complete clearing of the skin lesions was achieved in all five patients within 24.2 ± 3.2 days (X-linked 21.75 ± 6.5 , lamellar 23 days). Histopathology showed reduction of the hyperkeratosis, and thickening of the granular layer.

Clinical side effects were of mild intensity and included cheilitis, conjunctivitis and pruritus. All side effects were reversible upon reduction of the daily dosage.

In three patients treatment was discontinued after clearing of lesions. Fresh lesions re-appeared 6 weeks later.

One patient with X-linked ichthyosis developed two recurrences during maintenance treatment; one patient with lamellar ichthyosis was kept in complete remission for 9 weeks on a reduced daily dosage.

Ichthyosis is the generic term applied to a group of different disorders of keratinization all characterized by varying degrees of hyperkeratosis and scaling (Frost & Van Scott, 1966).

Vitamin A acid has a favourable effect on hyperkeratosis, and has been used in different hyperkeratotic conditions (Eriksen & Cormane, 1975; Orfanos *et al.*, 1973; Orfanos & Runne, 1975; Prutkin *et al.*, 1973). Symptoms of hypervitaminosis A limit dosage and duration of therapy. This fact has led to a search for derivatives with lower toxicity but comparable therapeutic efficacy. One such aromatic retinoid derivative, code number Ro 10/9359, (kindly provided for therapeutic trial by Hoffmann-La Roche, Vienna), exhibited a particularly good therapeutic ratio in animal experiments (Bollag, 1974). This paper presents the results of a therapeutic trial with Ro 10/9359 in five patients with ichthyosis.

PATIENTS (see Table 1)

Five patients, two girls and three boys, with ichthyosis were classified according to Frost & Van

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TABLE 1. Clinical data of patients

Patient	Age (years)	Sex	Weight (kg)	Type of ichthyosis	Time of onset	Clearing phase				Maintenance phase			
						Duration (days)	Daily dose (mg)	Response to therapy	Side effects	Duration (days)	Daily dose (mg)	Recurrence	Side effects
1	13	M	88	XLI	8 years	30	100	Very good	Cheilitis	50	0	Mild	0
2	17	M	53	XLI	Birth	22	50	Very good	Cheilitis	63	25	Severe	Cheilitis
						21	50	Very good	Cheilitis	59	25	Severe	Cheilitis
						14	50	Very good	Conjunctivitis cheilitis	10	25		
3	5	M	16	LI	Birth	23	25	Very good	Cheilitis pruritus	42	0	Mild	0
4	12	F	25	LI	Birth	23	25	Very good	Cheilitis pruritus	42	0	Mild	0
5	16	F	45	LI	Birth	23	50	Very good	Cheilitis pruritus	62	10	0	Mild cheil- itis

XLI = X-linked Ichthyosis, LI = Lamellar Ichthyosis.

Scott (1966) and selected for therapy. Three of them (Cases 3–5) were siblings with lamellar ichthyosis (LI); two unrelated boys suffered from X-linked ichthyosis (XLI).

Onset of the disease was at birth in all cases except one boy with XLI (Case 1) who developed the disorder during childhood. Due to the severity of their disease, all patients had been treated unsuccessfully by several topical and systemic therapeutic regimens. Patients were kept without specific therapy for 2 weeks prior to the onset of the study. Histologically, hyperkeratosis and a prominent granular layer were observed in all specimens. The number of mitoses (high in LI, few in XLI) served to distinguish these two forms from one another.

TREATMENT

The aromatic retinoid Ro 10/9359 (ethyl all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-1-2,4,6,8-nonatetraenoate) was provided in capsules containing 25 and 10 mg of the substance. The drug was administered orally according to body weight: initially, the patients received ≈ 1 mg/kg body weight, daily (details see Table 1). After complete remission, therapy was discontinued in the three younger patients. The two older cases were kept on a reduced dosage as maintenance therapy. During the initial phase of treatment all patients were hospitalized. They did not receive any local therapy at all.

Laboratory tests

The following laboratory tests were performed at weekly intervals (every 3 weeks during maintenance treatment): blood pressure, erythrocyte sedimentation rate, urinalysis, complete blood cell counts, clotting time, fasting blood sugar, SGOT, SGPT, alkaline phosphatase, LDH, serum bilirubin, serum electrolytes, serum iron, cholesterol, triglycerides, uric acid, blood urea nitrogen and serum creatinine determinations.

Six mm punch biopsies were obtained from involved buttock skin and repeated after clearing close to the previous biopsy sites.

RESULTS

Complete clearing of the skin lesions was achieved in all five cases. The mean duration to clearing was 24.2 ± 3.2 days (for LX 21.75 ± 6.5 days and for LI 23 days) ($\pm$ = standard deviation). After 2 weeks of treatment a mild erythema appeared and shedding of scales occurred beginning on the extensor surfaces of the extremities. Eventually, after clearing of lesions, slight hyperpigmentation and striae appeared at the predilection sites of the disease.

Maintenance treatment

To avoid possible side effects of hypervitaminosis A, such as growth disturbances, and to observe the duration of remission, therapy was discontinued after clearing of lesions in the 3 younger patients (Cases 1, 3, 4). They remained in complete remission for at least 6 weeks without any therapy at all. Subsequently slight scaling and hyperkeratosis started again at the previously involved sites.

The two older patients, one girl with LI (Case 5) and one boy with XLI (Case 2), were treated with a reduced dose (see Table 1), and showed a different course: the girl (Case 5) was kept completely free receiving 10 mg of Ro 10/9359 daily for 9 weeks. The pruritus she had suffered initially decreased, and finally disappeared without any additional treatment. In contrast, the boy with XLI (Case 2) experienced two recurrences within a period of 4 months on maintenance treatment. Both

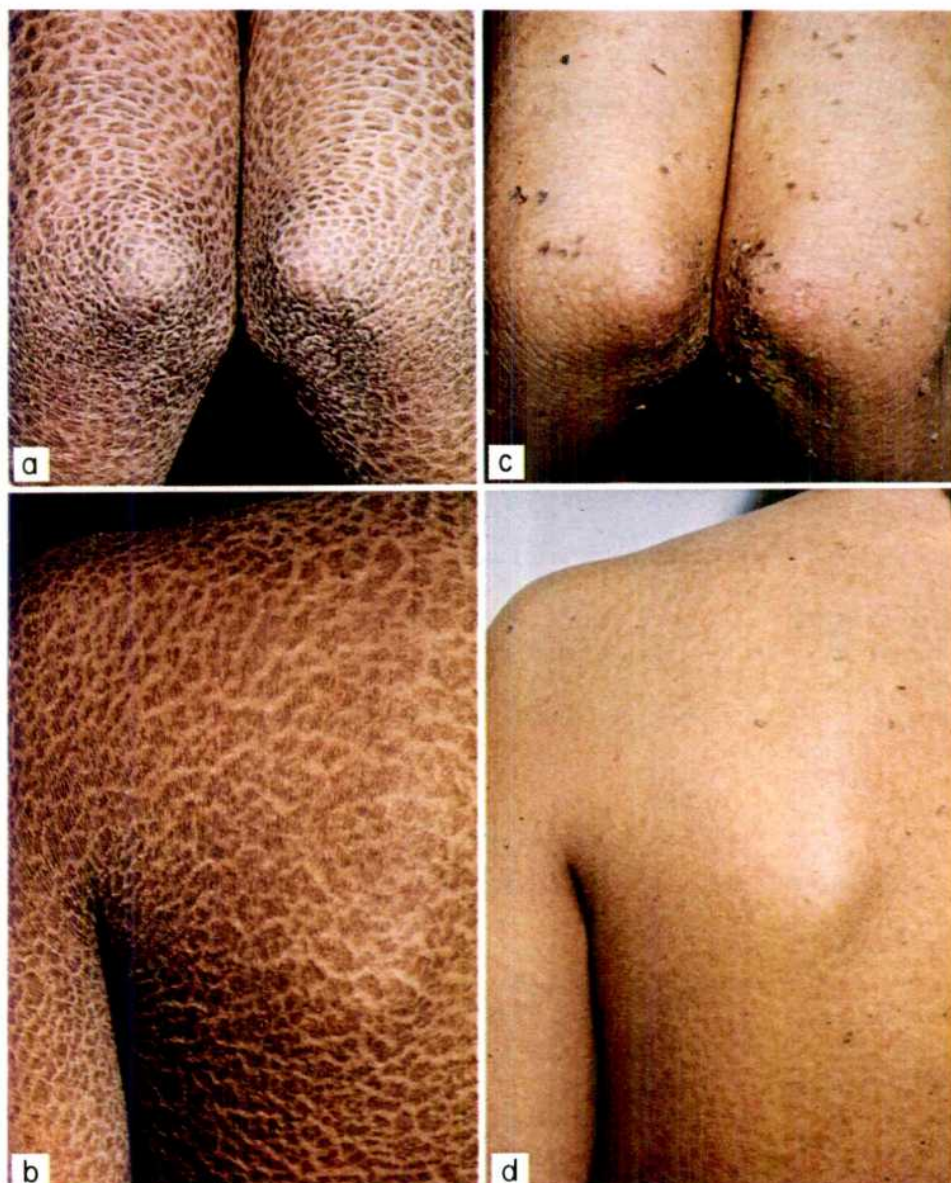


FIGURE 1. Patient no. 5 (female, age 16 years); (a) legs, (b) shoulder, before treatment; (c) legs, (d) shoulder, after treatment. Twenty-three days of oral aromatic retinoid (Ro 10/9359) were required for clearing.

recurrences were severe, but of lesser intensity than before treatment, and responded well to re-institution of higher dosage as used in the clearing phase. Complete remission was achieved again within 2 weeks.

Histopathological changes during therapy

All control biopsies obtained after the clearing phase showed histopathological differences when compared to the pretreatment specimens: The pronounced hyperkeratosis found in the untreated skin had disappeared and a normal stratum corneum was found in all patients. However, the granular layer appeared thickened in all slides as compared to the untreated skin.

Laboratory tests

The laboratory tests performed before and during treatment did not show any significant abnormalities. Only the alkaline phosphatase was slightly increased to 214 /ul in one patient (Case 3) during the initial treatment phase, but was found to be normal when the drug was discontinued.

Side effects

All patients developed cheilitis, which was treated without success with steroid free ointments, and remained a therapeutic problem. Reduction of dosage during maintenance phase improved the cheilitis. Pruritus was observed in three patients (only during the initial phase of therapy) and was treated with antihistamines. No erythema of the palms and soles, or other side effects as reported elsewhere (Ott, 1977), could be observed.

DISCUSSION

This clinical study demonstrates the therapeutic effect of the new aromatic retinoid Ro 10/9359 in two types of ichthyosis, the lamellar type and the X-linked type. All five patients responded equally well by complete remission. No significant differences in dosage were needed to clear the skin lesions nor was there a difference in duration of the initial clearing phase between XLI and LI. The time interval for reappearance of the skin lesions, when therapy was discontinued, seems to be the same in LI and XLI. The only difference noticed between the two forms was the appearance of recurrences during maintenance treatment. Case 5 suffering from LI could be kept completely free during maintenance therapy whereas Case 2 (XLI) showed two recurrences. Our preliminary results are at variance with those of other therapeutic trials (Ott, 1977), since side effects proved to be mild and did not force us to terminate treatment. Cheilitis is a problem, however, occurring in all cases and being unresponsive to local measures.

In summary, oral treatment with aromatic retinoids, such as Ro 10/9359, appears to be a promising therapy for ichthyosis, both of the LI and XLI types. Therapeutic efficacy can be achieved with a regimen of 1 mg/kg body weight. Side effects of systemic aromatic retinoids will have to be studied in future trials involving greater number of cases.

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REFERENCES

- BOLLAG, W. (1974) Therapeutic effects of an aromatic retinoid acid analogue on chemically induced skin papillomas and carcinomas of mice. *European Journal of Cancer*, **10**, 731.

- ERIKSEN, L. & CORMANE, R.H. (1975) Oral retinoic acid as therapy for congenital ichthyosiform erythroderma. *British Journal of Dermatology*, **92**, 343.
- FROST, P. & VAN SCOTT, E.J. (1966) Ichthyosiform dermatoses: classification based on anatomic and biometric observations. *Archives of Dermatology*, **94**, 113.
- HAPPLE, R., KASTRUP, W. & MACHER, E. (1977) Treatment of systematized verrucous epidermal nevus with oral application of a new retinoid. *Dermatologica*, **155**, 200.
- ORFANOS, C.E. & RUNNE, U. (1976) Systemic use of a new retinoid with and without local dithranol treatment in generalized psoriasis. *British Journal of Dermatology*, **95**, 101.
- ORFANOS, E.C., SCHMIDT, H.W., MAHRLE, G., GARTMANN, H. & LEVER, W.F. (1973) Retinoic acid in psoriasis: its value for topical therapy with and without corticosteroids. *British Journal of Dermatology*, **88**, 167.
- OTT, F. (1977) Behandlung der Psoriasis mit einem oral wirksamen aromatischen Retinoid. *Schweizerische medizinische Wochenschrift*, **107**, 144.
- PRUTKIN, L. (1967) The effect of vitamin-A acid on hyperkeratinization and the keratoacanthoma. *Journal of Investigative Dermatology*, **49**, 165.
- SCHIMPF, A. (1976) Zur systemischen Anwendung eines aromatischen Vitamin-A-Säure Derivates (Ro 10/9359) bei Psoriasis und Keratosen. *Zeitschrift für Haut-und Geschlechtskrankheiten*, **51**, 265.

Case Reports

Atopic dermatitis and congenital deafness

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SUMMARY

In a family with perceptive, non-progressive hearing loss several of the members suffered from atopic dermatitis. The proband had a severe atopic dermatitis and an extremely high IgE value. Some of the family members suffered from atopic dermatitis, others from deafness, and some from both diseases. The co-existence of these two disorders has been previously described in two families. Atopic dermatitis and perceptive, non-progressive congenital deafness might be genetically associated.

A number of hereditary syndromes with dermatological and auditory manifestations have previously been published (Reed *et al.*, 1967). Atopic dermatitis accompanied by congenital deafness has been reported in two families (Koningsmark *et al.*, 1968; Frentz *et al.*, 1976). We report our findings in a family where some of the members suffer from atopic eczema, others from deafness and some from both diseases.

MATERIAL AND METHOD

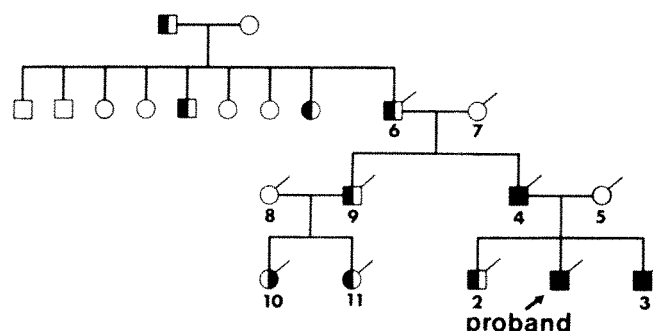
The material consists of 23 family members representing four generations. Eleven of them were examined, while information about the other family members has been obtained through questionnaires (Fig. 1).

The otological examination was supplemented with sound and speech audiometric tests, tympanometric tests and stapedius reflex tests. Furthermore, the proband was examined by a tone decay test, vestibular test and X-ray examination of temporal bones including tomography.

Laboratory investigations included haemoglobin, leukocytes, differential count, sedimentation rate, circulating eosinophils and immunoglobulins.

CASE REPORT

The proband is a 15-year-old boy who has suffered from atopic dermatitis since the age of 9 months. At first the eczema appeared on the cheeks; later on it was localized to the arms and legs especially involving the flexural areas. From early childhood he also suffered from asthma and allergic rhinitis. His eczema as well as the asthma and the rhinitis exacerbated during spring.



- ● male/female affected with atopic dermatitis
 ■ ● male/female affected with congenital deafness
 □ ○ male/female investigated

FIGURE 1. Pedigree of the family.

From the age of 3 years hearing loss was suspected. However, he was not audiological examined until he was 11-years-old. Before that time he had stayed at a children's psychiatric hospital for almost 3 years on the assumption that he was mentally retarded as he had a speech impediment.

He had a typical atopic dermatitis, with eczematous and lichenified lesions involving the hands, wrists, forearms, ankles and legs. Furthermore, excoriated papules were observed around the neck and on the upper trunk. There were fissures around the mouth and palmar keratosis with accentuated skin lines.

The audiometric test revealed a pronounced bilateral perceptive hearing loss (Fig. 2) with a 'basin curve' and small conductive element. Otoscopy showed a bilateral external otitis and a slightly thickened tympanum in the right ear. The tympanometric test showed a slight low-pressure in both middle ears. The other otological examinations were normal.

Laboratory investigations. Circulating eosinophils 867 mm^{-3} (normal range 0–450), IgG, IgM and IgA normal, IgE 30000 units/ml (normal range 0–150 units/ml, 1 unit = 2.3 ng/ml). Intracutaneous skin tests to common allergens were positive to the following antigens: house dust mite, birch, alder, hazel. Allergen specific IgE in serum (RAST, Pharmacia): house dust mite 4, birch 4, alder 4, hazel 3, dog 2, horse 2. Allergen specific IgE in serum measured by aluminium radio-allergosorbent test (Weeke, 1978): house dust mite 2319, birch 26, alder 17, hazel 7, dog 2, horse 2.

Chromosome study. Normal complement 46, XY. Chromatographic study of amino acid content in the urine showed normal levels.

His mental and linguistic development started to make progress after the beginning of audiological treatment and speech therapy. Intensive house dust clearance in the home, disodium cromoglycate given nasally and treatment with topical steroids improved the atopic diseases. During the improvement the IgE level dropped to 6800 units/ml.

Family history and findings

The numerical order of the 11 examined family members is shown in Fig. 1.

No. 3 (16-years-old). During infancy he had dry skin and itching eczema in the flexures. He still shows keratosis pilaris on both legs. A perceptive hearing loss was found at the age of 10 years with

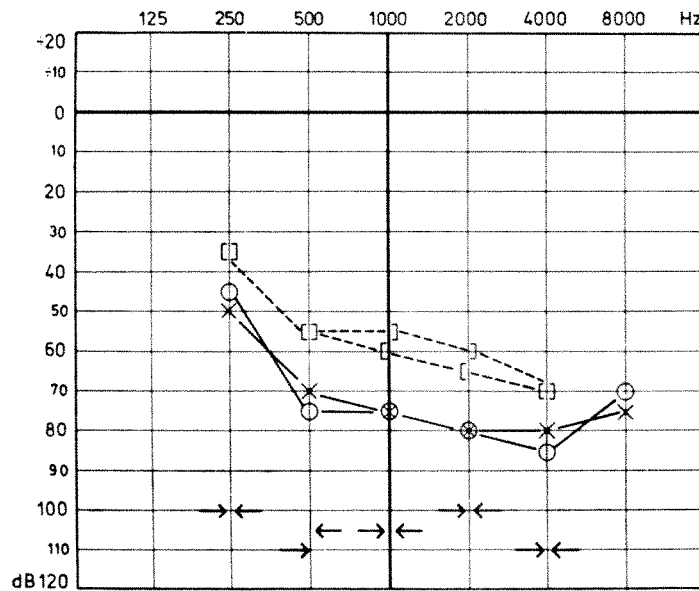


FIGURE 2. Audiogram of the proband. Symbols: air conduction, right ear (○—○); left ear (×—×). Bone conduction, right ear (]- - - -]); left ear ([- - - -]). Stapedius reflex, sound in right ear (→), sound in left ear (←).

35 decibels of the right ear and 50 decibels of the left. It was not possible to motivate the patient for audiological treatment.

No. 4 (40-years-old). During infancy he had a severe atopic dermatitis which had partly disappeared by the age of 14 years. Lichenified patches on the upper arms are still present. He has also had hearing loss since infancy with a bilateral, symmetrical perceptive hearing loss of 55 decibels. He was not motivated for audiological treatment.

No. 10 (16-years-old). Since the age of 2 years she has had eczema involving the flexor surfaces, treated for many years with steroid-containing ointments. She has normal hearing.

Nos 2 (10-years-old), 6 (82-years-old), 9 (40-years-old) and 11 (14 years-old). All of them suffer from severe bilateral perceptive hearing loss similar to that of the proband, but they do not have atopic dermatitis.

None of the examined family members have had asthma or hay fever. All blood tests were found normal including IgE. The paternal grandfather's siblings had 78 children and grandchildren, but according to the questionnaires none of these suffered from atopic diseases.

None of the examined intermarried women knew of atopy in their families. None of the examined parents was related.

DISCUSSION

In 1968 Koningsmark *et al.* published a study of a family where 3 of 4 siblings suffered from both congenital deafness and atopic dermatitis with a late onset. The parents, their siblings and the fourth brother did not have atopic dermatitis or hearing loss, and Koningsmark *et al.* (1968) suggested it to be a recessive disease.

Recently a family was reported (Frentz *et al.*, 1976) where two brothers suffered from atopic

dermatitis and congenital deafness. No further cases of hearing loss were present in the family, but the mother and the maternal grandfather both had atopic dermatitis during infancy and in the paternal family several members suffered from palmar and plantar hyperkeratosis.

Our proband had a severe atopic dermatitis. The atopic disease can be traced back in two generations and the hearing loss in four. The hearing loss in this family was found to be dominantly inheritable and of a perceptive, non-progressive type similar to that described in the two above-mentioned families (Koningsmark *et al.*, 1968; Frentz *et al.*, 1976). The prevalence of autosomal dominant hearing loss in a population is estimated at 0.03–0.003% (Brown, 1973). Atopic eczema is a common condition and could well be found by chance in a family with deafness. This might be supported by the lack of any eczema in the grandparents' generation. However, it seems to be more than chance to find congenital deafness transmitted from a father to his three sons and simultaneous transmission of atopic dermatitis to two of them. Furthermore one of the proband's female cousins suffered from atopic dermatitis, the other from congenital deafness. The accumulated cases in our family suggest that atopic dermatitis and congenital deafness might be genetically associated.

REFERENCES

- BROWN, K.S. (1973) Genetic features of deafness. *Journal of the Acoustical Society of America*, **54**, 569.
- FRENTZ, G., EVERBERG, G. & WULF, H.C. (1976) Congenital perceptive hearing loss and atopic dermatitis. *Acta Otolaryngologica*, **82**, 242.
- KONINGSMARK, B.W., HOLLANDER, M.B. & BERLIN, C.I. (1968) Familial neural hearing loss and atopic dermatitis. *Journal of the American Medical Association*, **204**, 95.
- REED, W.B., STONE, V.M., BORDER, E. & ZIPRKOWSKI, L. (1967) Pigmentary disorders in association with congenital deafness. *Archives of Dermatology*, **95**, 176.
- REED, W.B., STONE, V.M., BORDER, E. & ZIPRKOWSKI, L. (1967) Hereditary syndromes with auditory and dermatological manifestations. *Archives of Dermatology*, **95**, 456.
- WEEKE, B. (1977) *To be published.*

Yellow nail syndrome associated with mental retardation in two siblings

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SUMMARY

Two siblings with yellow nail syndrome and mental retardation are described. In addition to nail changes, the younger brother was found to have congenital lymphoedema and idiopathic pleural effusion, and the elder brother, lymphoedema praecox. They had in common a history of respiratory tract infections. Their intelligence quotient as measured by the Suzuki-Binet tests was 30% and 70%, respectively.

Since the first description of yellow nail syndrome by Samman & White (1964), a total of 69 cases of this rare condition have been reported up to 1976. This paper seems to be the first description of yellow nail syndrome associated with mental retardation.

CASE REPORTS

Case 1

M.K., a 26-year-old male. The patient was born in 1949. At birth he was in an asphyxial state, with his scrotum and the lower part of his abdomen markedly swollen. At the age of 2, mental retardation became evident. At age 5 he was admitted to a hospital for treatment of pleurisy. At age 7 or 8, deformity of the nails became evident. The swelling of the scrotum and lower part of the abdomen became increasingly larger, and at age 13 lymphorrhoea from the enlarged scrotum began to afflict the patient. He came to our clinic in January, 1976 with oedematous swelling of both feet, 4 months for the right foot and 5 months for the left foot.

Family history revealed close consanguineous marriages in his family. His paternal and maternal grandfathers were siblings and the grandmothers also siblings. His parents are therefore cousins. His only sibling is an elder brother who is Case 2 in this paper. None of his other family members is known to have oedema or mental retardation.

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Physical examination showed a vacant expression and distinct dorsal scoliosis. On auscultation breath sounds were diminished over the right chest. Swelling was prominent over the area extending from the lower part of the abdomen to the scrotum, which was markedly enlarged and hard without pitting. The penile skin was thickened. The lower extremities were oedematous (Fig. 1). All the

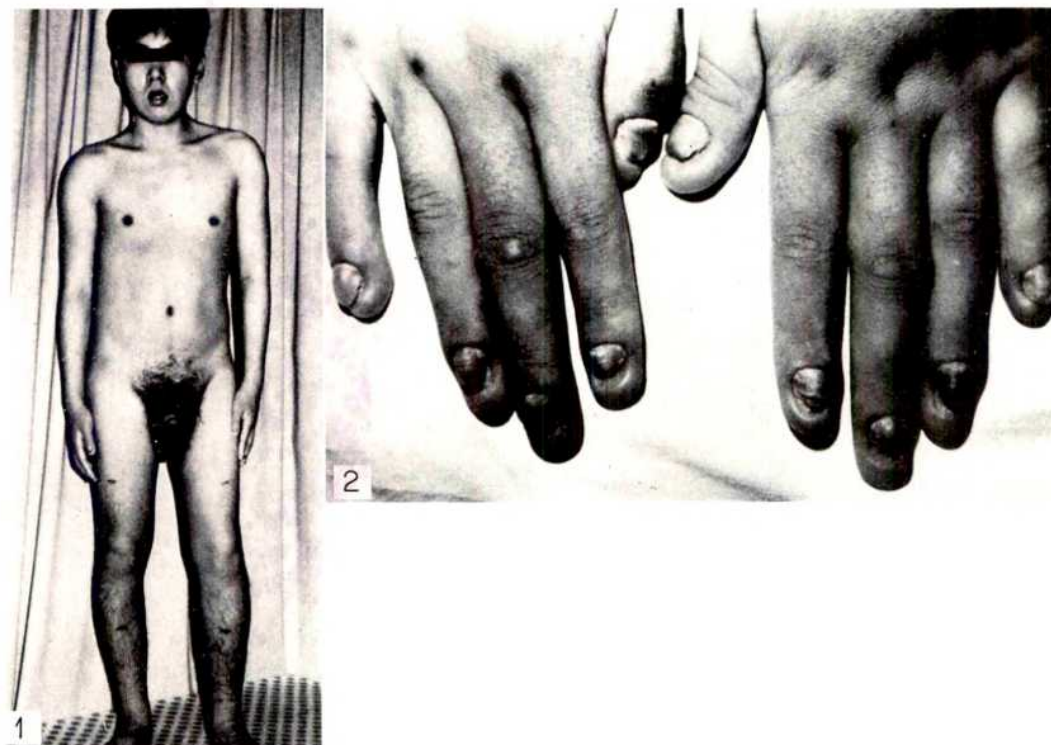


FIGURE 1. Case I. Lymphoedema of lower half of body.

FIGURE 2. Case I. Deformed fingernails.

fingernails and toenails were pale yellow in colour, thickened, stunted, and showed excessive side-to-side curvature. Their surfaces were roughened and slight transverse ridging was evident. There was also onycholysis, absence of the lunulae, and loss of cuticles (Fig. 2).

Examination showed an intelligence quotient as measured by the Suzuki-Binet test of 30%; chromosome analysis, blood count, urinalysis, serum chemistry, erythrocyte sedimentation rate, serum electrophoresis, immunoelectrophoresis, and ECG were all normal. The tuberculin reaction was negative. Fingernails were negative for infecting microorganisms, and midnight blood was also negative for *Microfilariae Bancrofti*. Lymphangiography was successful on the right foot alone. In the right thigh the contrast medium made visible two very small lymphatics which drained into the large right inguinal lymph node, and refused to travel toward the external iliac lymph node. Forty-eight hours later the medium made visible a lymphatic channel running from the lateral side of the greater trochanter upward through the subcutaneous tissue on the right lateral side of the trunk (Fig. 3). Chest X-ray showed subcutaneous lymph nodes in the right axillary region and suggested the formation of unusual collateral lymphatic channels running upward from the lower to the upper



FIGURE 3. Lymphangiogram of right groin in Case 1, 48h after injection of contrast medium. Arrow shows unusual collateral circulation.

half of the body. Chest X-ray also disclosed scoliosis of the thoracic vertebrae and, on the right side, a high degree of pleural thickening and a small pleural effusion, which defied aspiration.

A reducing operation with omental transposition was performed on the scrotum. At operation the scrotal skin was thickened and the subcutaneous tissue was oedematous. No hydrocoele was detected. The postoperative course was uneventful, lymphorrhoea ceased, and the use of elastic stockings and diuretics has kept the lower extremities in satisfactory condition.

Case 2

N.K., a 30-year-old male. He was born in 1946. Although the patient did not exhibit any abnormalities at birth, his mental retardation became evident early in childhood. At the age of 10 deformity of the nails began to develop. Swelling occurred in the left foot at the age of 21 and in the right foot several years later, with repeated episodes of cellulitis. In the meantime he complained of prolonged productive cough and was diagnosed as having bronchitis at age 21. He was first seen by us in July, 1976.

Physical examination showed swelling of the lower extremities, marked below the knee, with the skin of the affected areas brownish and non-pitting. Fingernails and toenails resembled those in Case 1 but their surfaces retained more smoothness. Intelligence quotient was 70%. Chest X-ray was normal.

DISCUSSION

Yellow nail syndrome was first reported by Sammar & White (1964) who defined it as a condition

characterized by concurrence of yellow nails and primary lymphoedema. Two years after their description Emerson (1966) added idiopathic pleural effusion to the dyad on the supposition that idiopathic pleural effusion is also due to impaired lymphatic drainage. In later years Zervas (1965) and Dilley *et al.* (1968) reported cases of yellow nail syndrome associated with bronchiectasis. Bowers (1969) observed a similar case and surmised that bronchiectasis could be attributed to obstruction of bronchial lymphatics. In succession to these descriptions, studies led to a contention that the pathogenesis underlying respiratory tract infections seen in patients with lymphoedema, e.g. sinusitis, bronchitis, etc. might be accounted for by a deficiency of lymphatic drainage (Meiers *et al.*, 1973). Currently, clinical manifestations of patients who have been diagnosed as having yellow nail syndrome are varying combinations of the aforementioned triad and symptoms of respiratory tract infections (Otsuki, Sano & Kawashima, 1976).

The range of reported disorders manifested by the yellow nail syndrome is thus being expanded. Primary lymphoedema is known to be associated with various types of malformation (Kinmonth, 1972). Our scrutiny of the 69 reported cases of this syndrome has shown them to be associated with a variety of disorders other than the triad and respiratory tract infections, as shown in Table 1. Whether

TABLE 1. Disorders reported in patients with yellow nail syndrome, other than the triad and manifestations of respiratory tract infections

Hypertension	5
Myocardial disease	4
Hypogammaglobulinaemia	1
Hypoproteinaemia	3
α_2 -Macroglobulinaemia	1
Macroglobulinaemia (IgM)	1
High titres of rheumatoid factor	1
Rheumatoid arthritis	1
Glomerulonephritis	1
SLE	1
Lymphopenia	1
Hodgkin's disease	1
Malignant melanoma	1
Anaplastic malignant tumour	1
Fibroma	1
Familial tremors	1
Loss of hearing	1
Glaucoma	1
Ocular hypertelorism	2
Thrombophlebitis	1
Varix	1
Thyroid tumour	1
Hashimoto's disease	1
Hypothyroidism	1
Unequal breasts	1
Congenital luxation of hip joints	1
Hyperflexibility of wrist joints	1
Diabetes mellitus	1
Hepatitis	2
Pulmonary infarction	1

all of them are related to impaired lymphatic drainage remains uncertain. Beninson *et al.* (1974) suggested that lymphatic dysplasia might induce systemic disorders affecting multiple organs. Hiller, Rosenow & Olsen (1972) suggested that the concurrence of Hodgkin's disease, malignant melanoma, and anaplastic malignant tumour was more fortuitous. Thus any clinical features seen in patients with yellow nail syndrome should be carefully noted for future work. No paper but ours has reported mental retardation as a complication of yellow nail syndrome. Although whether or not mental retardation is actually related to defective lymphatic drainage remains uncertain, the family history of our subjects strongly suggests that some unknown genetic factors are involved in the development of coexisting mental retardation and impaired lymphatic drainage.

REFERENCES

- BENINSON, J., RIAN, R.L., EYLER, W.R. & DUSALT, L.A. (1974) The renogram in lymphatic dysplasia. *Lymphology*, **3**, 123.
- BOWERS, D. (1969) Unequal breasts, yellow nails, bronchiectasis and lymphedema. *Canadian Medical Association Journal*, **100**, 437.
- DILLEY, J.J., KIERLAND, R.R., RANDALL, R.V. & SHICK, R.M. (1968) Primary lymphedema associated with yellow nails and pleural effusion. *Journal of the American Medical Association*, **204**, 670.
- EMERSON, P.A. (1966) Yellow nails, lymphoedema, and pleural effusions. *Thorax*, **21**, 247.
- HILLER, E., ROSENOW, E.C., III. & OLSEN, A.M. (1972) Pulmonary manifestation of the yellow nail syndrome. *Chest*, **61**, 452.
- KINMONTH, J.B. (1972) *The lymphatics: Diseases, Lymphography and Surgery*. Edward Arnold, London.
- MEIERS, H.G., GREUEL, H., PERSCHMANN, U., JOST, G., HUBBES, A. & GREITHER, A. (1973) Yellow nail syndrome. *Deutsche Medizinische Wochenschrift*, **98**, 1829.
- OTSUKI, O., SANO, T. & KAWASHIMA, Y. (1976) Yellow nail syndrome: Report of two cases. *Japanese Journal of Clinical Dermatology*, **30**, 431 (Japanese).
- SAMMAN, P.D. & WHITE, W.F. (1964) The 'yellow nail' syndrome. *British Journal of Dermatology*, **76**, 153.
- ZERFAS, A.J. (1965) Yellow nail syndrome with bilateral bronchiectasis. *Proceedings of the Royal Society of Medicine*, **59**, 448.

Non-inflammatory dermal elastolysis

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SUMMARY

A 33-year-old woman is described who suffers from an idiopathic loss of mid-dermal elastic tissue which leads to wrinkling of the skin and to discrete perifollicular protrusions. In accordance with Shelley & Wood (1977) we conclude that these findings represent a new entity for which we propose the term 'non-inflammatory dermal elastolysis'.

This report deals with the skin changes in a woman exhibiting a distinctive disease process, which appeared clinically as widespread, well defined areas of wrinkles of the skin and areas consisting of discrete perifollicular papules. The skin changes appeared slowly and progressively without any symptoms or accompanying disease. The disorder is characterized histologically by a specific mid-dermal loss of elastic tissue.

CASE REPORT

A 33-year-old, well nourished and well developed white woman noticed a plaque-like circumscribed area of unusual thinning of the skin of her left upper arm 4 years ago. The changes slowly spread over the shoulders, the right upper arm, the abdomen and areas of the back, leading to a misfitting aged appearance of her skin. The face and legs were uninvolved.

The lesions consist of well defined areas exhibiting two morphological patterns: Type I lesions (Fig. 1a), small, tiny wrinkles arranged parallel to the skin cleavage lines; Type II lesions (Fig. 1b), miniature, soft papular lesions consisting of tiny perifollicular protrusions, leaving the hair follicle as an indented centre. The affected skin areas are asymptomatic, with normal skin colour. There are no signs of atrophy or inflammation.

Past history revealed tonsillectomy in childhood and pyelonephritis at the age of 16. There was no history of urticaria or any other inflammatory or non-inflammatory skin disorders. The patient's family is in good health; there is no consanguinity of her parents.

The general physical examination, ophthalmological examination, tests for function of the lungs

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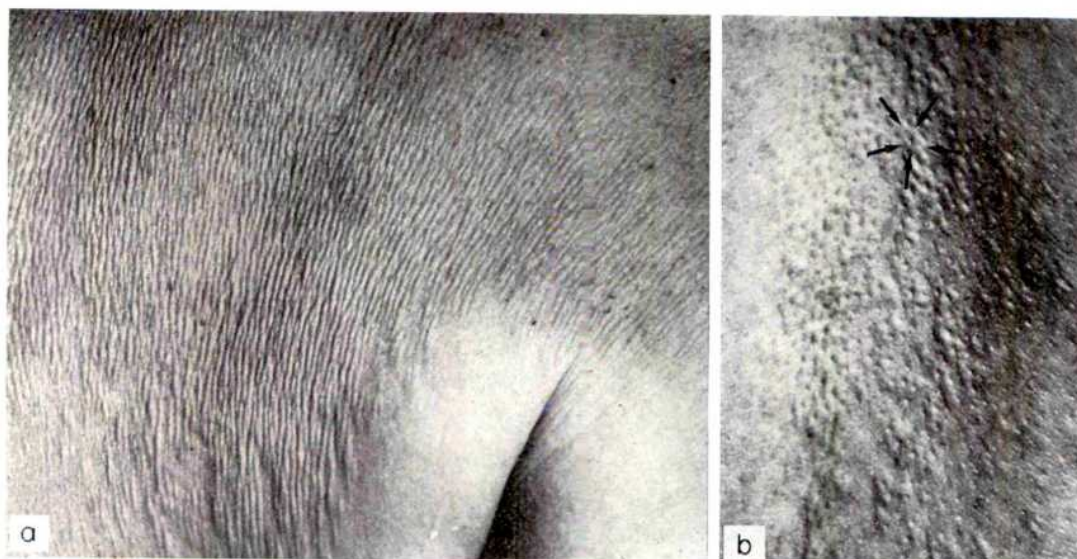


FIGURE 1. (a) Type I lesion: parallel fine wrinkles on right shoulder. (b) Type II lesion: small perifollicular protrusions (arrows) in sacral area.

and peripheral arteries, roentgenograms of oesophagus, stomach and bowel showed no abnormalities.

ESR, urinalysis, complete blood count, serum analysis for glucose, BUN, creatinine, sodium, potassium, chloride, calcium, phosphate, uric acid, iron, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, copper, IgG, IgM, IgA and C₃ were within normal ranges; VDRL and ANA were negative.

Skin biopsies, taken from both morphological variants as well as from normal appearing skin at the border of the affected plaques, were snap frozen for Sudan stain and direct immunofluorescence (Holubar, Stingl & Albin, 1976) and in addition embedded in paraffin as well as in EPON 812 for electron microscopy employing established methods of our laboratory (Gschnait, Wolff & Konrad, 1975). Haematoxylin and eosin stains showed no abnormalities of the epidermis and dermis. Connective tissue, blood vessels, sweat glands, sebaceous glands and subcutaneous fat were apparently normal.

Weigert's elastica stain from type I lesions showed (Fig. 2a) a patchy loss of elastic tissue in the mid-dermis including elastic fibres of blood vessels, whereas elastic fibres in the papillary (Fig. 2a, inset) and deep reticular dermis appeared unchanged. The biopsy from a type II lesion revealed (Fig. 2b) similar features but the loss of elastic tissue was found throughout the total mid-dermal zone and was interrupted only by hair follicles which were engulfed by a thin layer of histologically intact elastic tissue. The elastic fibres at the border of the lesions did not appear disarranged or fragmented. Unaffected skin appeared normal.

Alcian blue stain revealed a normal distribution of acid mucopolysaccharides; toluidine blue stain demonstrated a regular amount of perivascular mast cells. Van Gieson, Gomori, PAS and Sudan stains showed no abnormalities in specimens from affected or unaffected skin. Direct immunofluorescence showed no abnormal deposits of IgG, IgM, IgA, IgD and complement in affected and unaffected skin areas.

By electron microscopy the epidermis and the structural components of the dermo-epidermal junction zone appeared normal. There was a marked reduction of elastic fibres in the mid-dermal

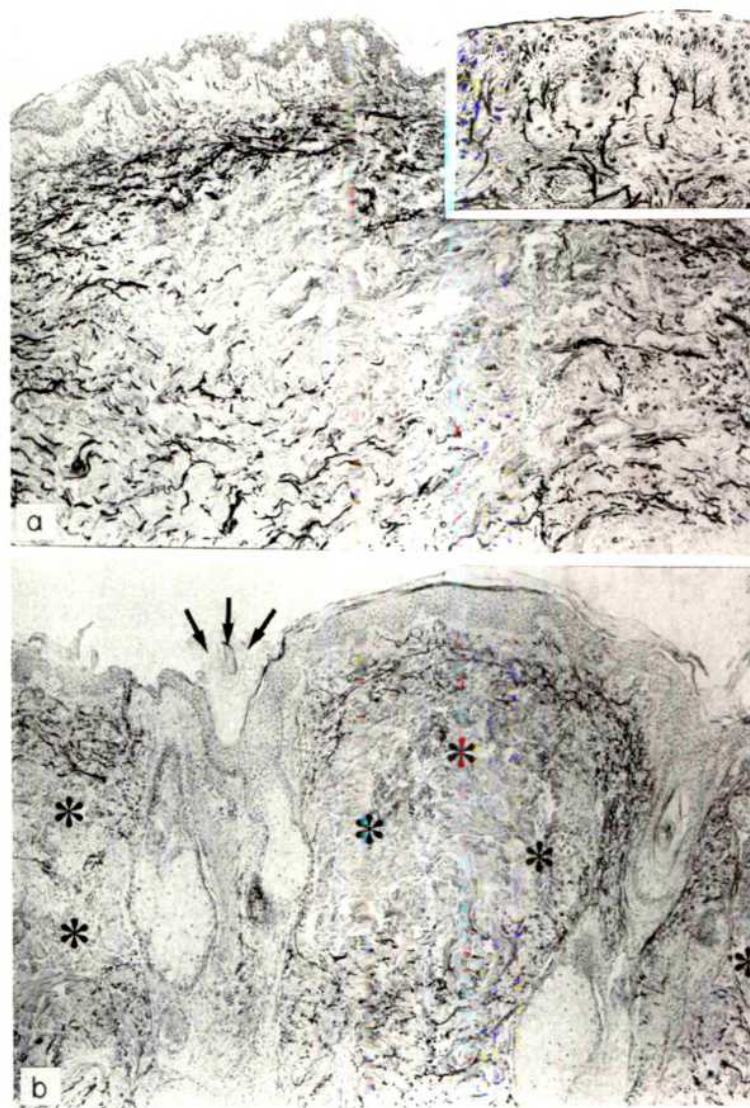


FIGURE 2. (a) Type I lesion: patchy loss of elastic tissue in the midportion of the dermis. Inset demonstrates that the subepidermal elastic tissue remains histologically normal (Weigert's elastica stain, $\times 16$; Inset, $\times 40$). (b) Type II lesion: loss of elastic tissue throughout the total mid-dermal zone (asterisks) interrupted only by hair follicles. The interfollicular skin seems to protrude, the hair follicles appear retracted (arrows) ($\times 16$).

zone corresponding to the histological findings. The other dermal constituents including collagen fibres, vessels and fibroblasts showed no abnormalities.

DISCUSSION

The clinical hallmark of this most unusual case is fine wrinkling of the skin and tiny perifollicular papules both confined to circumscribed areas. These changes correlate histologically to a loss of

elastic tissue in the mid-dermis, whereas the elastic tissue of the papillary and deep dermis remains apparently normal.

Shelley & Wood (1977) recently described the first observation of 'wrinkles due to idiopathic loss of mid-dermal elastic tissue' in a 42-year-old woman. The clinical and histological features closely resemble the findings in our patient and thus these two cases can be considered as an identical disease process. Shelley & Wood, however, interpreted their case as a possibly secondary, post-inflammatory process due to previous attacks of urticaria. Our patient gave no history of any preceding inflammatory or non-inflammatory skin disease and clinical, histological and electron microscopic examination revealed no signs of an accompanying or underlying disorder. Thus we have to conclude this is a primary idiopathic process.

Our patient exhibited two distinct clinical patterns; wrinkles, which were already described by Shelley & Wood, and in addition perifollicular protrusions. These two patterns corresponded to different histological findings. A possibly channel-like loss of elastic fibres in the mid-dermis, which in cross sections appears as a patchy lack of elastic tissue, may lead to linear protrusions of the overlying skin, thus forming wrinkles arranged in parallel. The diffuse loss of elastic tissue throughout the whole mid-dermis, interrupted only by hair follicles, which themselves are engulfed by well preserved elastic structures, may in turn explain the morphology of the type II lesions (interfollicular papular protrusions leaving the hair follicles as depressed centres).

The morphological and histological differential diagnosis of this peculiar disease has been discussed in detail by Shelley & Wood (1977). The skin changes are somewhat similar to the post-inflammatory elastolysis (Verhagen & Woerdemann, 1975), which was observed in young Africans. This disorder, however, is characterized by the complete absence of elastic tissue in the upper and mid-dermis and by the fragmentation of the elastic fibres leaving granular remnants. In contrast to our case, post-inflammatory elastolysis starts with an acute phase of firm, erythematous lesions.

The present case is characterized by a peculiar lack of elastic tissue in the mid-dermis leading to two different patterns of clinical appearance. There are no other outstanding pathological details, no changes in epidermis, hypodermis or in the skin appendages and no preceding inflammatory process. In summary, in accordance with Shelley and Wood, we conclude that these cases represent a new entity for which we propose the term 'non-inflammatory dermal elastolysis'.

REFERENCES

- GSCHNAIT, F., WOLFF, K. & KONRAD, K. (1975) Erythropoietic protoporphyria. Submicroscopic events during the acute photosensitivity flare. *British Journal of Dermatology*, **92**, 545.
- HOLUBAR, K., STINGL, G. & ALBINI, B. (1976) Praxis und Methodologie der definierten Immunfluoreszenz. *Hautarzt*, **27**, 78.
- SHELLEY, W.B. & WOOD, M.G. (1977) Wrinkles due to idiopathic loss of mid-dermal elastic tissue. *British Journal of Dermatology*, **97**, 441.
- VERHAGEN, A.R. & WOERDEMAN, M.J. (1975) Post-inflammatory elastolysis and cutis laxa. *British Journal of Dermatology*, **92**, 183.

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SIR, Further to my report of *Cutis Marmorata telangiectatica* (*British Journal of Dermatology* 1977, **97**, 437) I wish to advise of the following development.

The patient had a series of epileptic attacks on 8 August 1977, lost consciousness and died the following day. There was no previous history of epilepsy in her or her family.

Postmortem showed that death was due to subdural haemorrhage and the pathologist noted several areas of dura mater each about 3 cm in length with dilated veins similar to those on the abdominal wall.

Macroscopically and microscopically no other abnormality was shown.

It seems likely that rupture of a dilated vessel in the brain caused this girl's death, although this type of cutaneous vascular abnormality is not usually associated with internal anomalies or haemorrhage.

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Generalized eczéma craquelé: a marker of zinc deficiency?

Recently, Barker & Cotterill (1977) reported generalized eczéma craquelé as a presenting feature of Hodgkin's disease in two patients. They did not reach any definite conclusion as to the possible aetiology of the peculiar eruption. Apparently it was not due to essential fatty acid deficiency or myxoedema. In both patients the dermatosis remained unresponsive to liberal application of emollients and corticosteroids.

Within the last 2 years, chronic zinc deficiency has been found in chronic alcoholics with malabsorption and gastric resections (Weismann *et al.*, 1976; 1978b). Such patients present hair and skin changes as seen in chronic stages of acrodermatitis enteropathica. Moreover, they all show pronounced generalized eczéma craquelé. The response to zinc supplementation is prompt and impressive. Apparently eczéma craquelé also occurred in acrodermatitis enteropathica before the era of zinc treatment. Although this was never encountered as a characteristic finding of this disorder, it might be due to ignoring 'uncharacteristic' skin changes. We have photographs from 1968 of an acrodermatitis patient with marked eczéma craquelé on limbs and back. This occurred during an exacerbation of the disease due to omitting the prescribed hydroxyquinoline therapy. It gradually subsided, together with other skin symptoms, as the quinoline therapy was continued under controlled conditions in hospital. Today, her skin is completely normal.

The question arises whether the two patients reported by Barker & Cotterill had conditional zinc deficiency. In one of the patients it seems conceivable as there was a 12-year history of coeliac disease which may lead to zinc deficiency (Elmes, Golden & Love 1974; Solomons, Rosenberg & Sandstead 1976). In both patients reported, local corticoid therapy was unsuccessful, as also in clearing skin changes of acrodermatitis and of zinc depletion following zinc-free long-term intravenous feeding. The eczéma craquelé of patients with conditional zinc deficiency remains unaffected by local therapy. This unresponsiveness shows, as also pointed out by Barker & Cotterill, that the generalized form of eczéma craquelé is another skin disorder than the form commonly seen among elderly patients especially during the cold seasons.

In conclusion, it is suggested that patients showing widespread recalcitrant eczéma craquelé should be investigated for a possible underlying zinc deficiency. A short therapeutic trial with zinc may be performed to substantiate or reject the diagnosis.

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REFERENCES

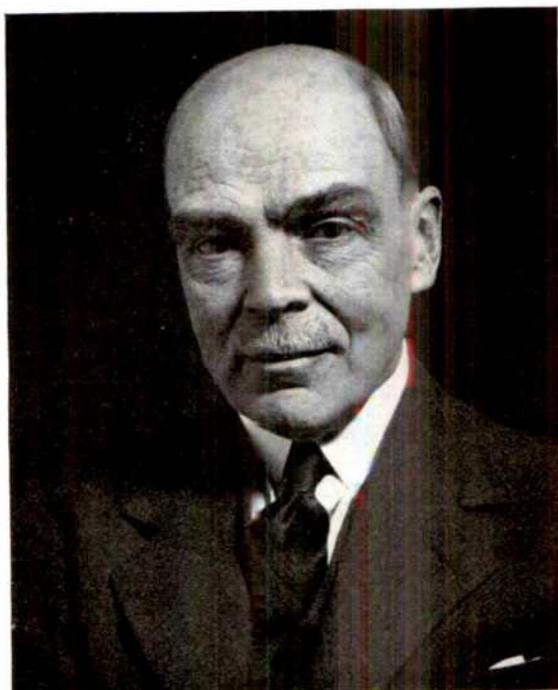
- BARKER, D.J. & COTTERILL, J.A. (1977) Generalized eczéma craquelé as a presenting feature of lymphoma. *British Journal of Dermatology*, **97**, 323.
- ELMES, M., GOLDEN, M.K. & LOVE, A.H.G. (1974) Unresponsive coeliac disease. *Quarterly Journal of Medicine*, **45**, 696.
- SOLOMONS, N.W., ROSENBERG, I.H. & SANDSTEAD, H.H. (1976) Zinc nutrition in coeliac sprue. *The American Journal of Clinical Nutrition*, **29**, 371.
- WEISMANN, K., ROED-PETERSEN, J., HJORTH, N. & KOPP, H. (1976) Chronic zinc deficiency syndrome in a beer drinker with a Billroth II resection. *Journal of International Dermatology*, **15**, 757.
- WEISMANN, K., WANSCHER, B. & KRAKAUER, R. (1978a) Oral zinc therapy in geriatric patients with selected skin manifestations and a low plasma zinc level. *Acta dermatovenereologica*, **58**, 157.
- WEISMANN, K., WADSKOV, S., MIKKELSEN, H.I., KNUDSEN, L., CHRISTENSEN, K.C. & STORGAARD, L. (1978b) Acquired zinc deficiency dermatosis in man. *Archives of Dermatology*, in press.

Obituary

DR JOHN FERGUSON SMITH

Dr John Ferguson Smith, one time head of the Department of Dermatology at Glasgow Royal Infirmary and President of the British Association of Dermatology 1958-9, died at his home in Strathtay, Perthshire on 29 April 1978 in his 90th year.

J.F.S. was born in East Ham on 11 August 1888. His father, curate in the Church of the Ascension, came of Edinburgh stock, whilst his mother belonged to the McKenzie family of Ballone and Inverbroom. When his parents moved to Glasgow in 1894 he attended the High School of Glasgow where he modestly claimed an undistinguished career. However, he obtained the necessary qualifications for University entrance in English, Latin, Greek, Mathematics and German at the age of 15. A year later he entered



Glasgow University where on his father's advice he took an arts course to teach him to study on his own and by combining this with pre-clinical science he was able to graduate M.A. in 1907 and M.B. Ch.B. in 1911. He had formed the ambition to be a doctor at the age of 12 and now set his sights at being a consultant in General Medicine. Immediately after qualifying he did a year in general practice at Auchinleck in Ayrshire. Here he acquired a knowledge of miners and their way of life which induced a feeling of sympathy with them which remained with him ever after.

He returned to Glasgow to become house physician to R. Barclay Ness for 6 months at the Western Infirmary followed by three months in casualty and three months in the skin wards. During this period, at his own request, he began bacteriological research with Professor Carl Browning from which arose a lifelong close friendship.

He then decided he should see some more of the world and signed on as a ship's doctor with the Blue Funnel Line for a year's experience on the China run. On arrival there he transferred at the company's request to an immigrant ship from China to the U.S.A. He visited many ports in Japan, the Eastern

Seaboard of North America, the Chinese coast, Indo-China, Singapore, Malay and on the way home Jeddah. He arrived home to find that he had been awarded a £100 research scholarship and resumed his work with Professor Browning until the outbreak of war in August 1914.

He at once volunteered and in September was sent with thirty others to set up a base hospital in Northern France. In May 1915 he was transferred to the 8th London (Howitzer) Brigade RFA with whom he served throughout the battles of Loos, Somme and Messines, being mentioned in dispatches. In September 1917 he was again transferred, this time to the 26th General Hospital at Étaples as pathologist. Because the surgical unit in this hospital had its own pathologist, he was not fully occupied and volunteered to look after 180 skin beds taking cases from a whole army area. This was the start of his dermatological career and also family life because here he met and shortly after married Miss E.M. Thorne who was working as a V.A.D.

He was demobilised in June 1919, purchased a small general practice in Partick in Glasgow and worked at it for three years. Once more, however, he had insufficient to keep his active mind busy and therefore restarted his bacteriological research at the Western Infirmary and at the same time became assistant to Dr George McIntyre, who was in charge of the Skin Department at Glasgow Royal Infirmary. When the latter died in 1921 J.F.S. was invited to replace him and he took over in 1922. He sold his general practice to a man who became another lifelong friend and the family doctor. He moved to 17 Woodside Place, at one time the home of Lord Lister, and settled down to consult in dermatology. Through his appointment at the Royal Infirmary he became lecturer in dermatology to Glasgow University and started an association which he maintained until he retired from the Royal in 1948. In 1926 as was the practice for dermatologists in those days he undertook the treatment of venereal disease for Glasgow Corporation, at the Broomielaw Clinic which grew up around him and his great friend and colleague Dr Herbert Brown. He retired from that establishment in 1953 having been the leading figure in its becoming a renowned centre of excellence for its treatment.

A founder member of the British Association in Dermatology in 1921 in all but name (he had not attended the dinner preceding the inaugural meeting) he became President in 1958-59 and an honorary member thereafter. Along with Sir Norman Walker and Sir Robert Bolam he started the North British Dermatological Society as an informal clinical meeting. This grew steadily over the years and was the forerunner of the present Scottish Dermatological Society. In 1936 he was awarded the M.D. with honours and his thesis on the bacteriology of sycosis barbae gained him the Bellahouston Gold Medal in 1937. He became F.R.F.P.S.(G) in 1949 and F.R.C.P.(G) in 1962. He represented Scotland on the Committee of the International Congress of Dermatology in London in 1952. For many years he was Scottish representative on the Dermatological Committee of the British Medical Association and because this entailed frequent visits to London he became a member, then vice-president and finally honorary member of the Section of Dermatology of the Royal Society of Medicine. He was also an honorary member of the German Dermatological Association.

J.F.S. had great dignity but was the most approachable of men. He was full of kindness and feeling for his fellow men. At the same time he enjoyed a fine sense of wit and humour but never to the embarrassment of those around. To his students and clinical assistants he was a legend in his life-time. He was a splendid clinical teacher and was in his element explaining the clinical details of disease or the intricacies of the histopathology of the skin of which he was an acknowledged expert. Although he addressed the students year in and year out in his course of lectures he preferred teaching in the clinic and at the bedside. He had a prodigious knowledge of his subject and could pin-point obscure references from memory with astonishing accuracy. Nothing was too much trouble for him. His junior colleagues frequently had reason to be grateful for his assistance and encouragement. This was particularly applicable to those who, like the undersigned, joined his department as a complete stranger but quickly came to feel the warmest personal regard for his kindness and hospitality.

He deservedly enjoyed a reputation far beyond the confines of Glasgow. He was not only well known and beloved by his colleagues in the rest of Britain but was highly regarded in both Europe and America. He had many close friends amongst European doctors and not only dermatologists. He had the gift of making and keeping friends wherever he went.

His name will be remembered in dermatology for decades to come because of his papers on familial multiple self-healing epithelioma, known universally as Ferguson Smith's disease. When he first described this rare condition he was disbelieved outside Scotland but so ably did he argue his case that he converted all his critics both at home and abroad. He published many other papers in dermatological journals and took an active part in technical discussions at Society meetings which he particularly enjoyed attending. Along with Herbert Brown and Alison McLaughlan he formed a formidable team who put Glasgow quite firmly on the dermatological map. With his great clarity of expression J.F.S. was an outstanding figure at any dermatological conference.

His interests extended far beyond the realms of medicine. He held a deep religious conviction and his faith sustained him in many difficult periods. He could discourse by the hour on the arts, music, photography and sport. He was particularly fond of music. With his keen ear and profound knowledge of orchestral music he was an enthusiastic devotee of the many splendid concerts given in Glasgow by the Scottish National Orchestra. He enjoyed the company of other people both at meetings but especially in his home where he was a hospitable and extraordinarily entertaining host. He was the living embodiment of the value of a broad education in the arts, literature and the classics to a doctor. He exercised his body as well as his mind. In his youth and for over 20 years after he was a keen cricketer and an able performer. During his period at the Royal Infirmary he walked the two miles from his home and back again every day always arriving at the same time with the regularity of a clock.

J.F.S. had a very happy family life and a devoted family. He and his wife enjoyed almost 60 years of married life. She was a source of great strength to him during several serious illnesses in his latter years. She survives him with happy memories of a marvellous man. All his four children were doctors. His older son John is consultant dermatologist at the Victoria Infirmary Glasgow whilst Malcolm is Professor of Genetics at the University of Glasgow.

The Glasgow school of dermatology owes a great deal to Jack Ferguson Smith. It will never be quite the same now that he has gone but likewise he will never be forgotten.

J.O'D. ALEXANDER

Book Reviews

Nasal Allergy. By NIELS MYGIND (1978) Oxford: Blackwell Scientific Publications Ltd. Pp. 373. B/w illus. Price £18.00.

Dermatologists not infrequently have to take an interest in allergic rhinitis, perhaps en passant while treating other atopic disorders, or because they are often involved in any form of skin testing. It is very interesting to read how we share comparable problems with ENT specialists—the multiplicity of factors, constitutional, immunological and non-immunological which confuse the assessment of ‘allergic’ rhinitis and of atopic dermatitis.

This book is written by an ENT specialist with a strong leaning to immunology. It is able to discuss basic immunology for the unsophisticated ENT surgeon and basic facts about the nose for the unsophisticated allergist, and to go into enough detail to please both camps. It is well suited to the needs of other disciplines and the references are commendably recent.

Relatively few allergens are given full consideration and this is not a book in which to find details of when and where to track down obscure causes of trouble. The evidence against common allergens like the house dust mite is given fully.

Can much that is written be extrapolated to or from dermatology—the role of ingested allergens; local production of IgE so that blood and other tissue levels may be merely an overspill; the significance of late onset IgE mediated skin tests and nasal provocation tests? What about the interesting correlation between the number of eosinophils in nasal secretion and response to topical steroid or disodium cromoglycate?

Skin tests emerge as valuable weapons, and the limitations of nasal and bronchial provocation tests and of laboratory tests are emphasized. The size of a positive test correlates very poorly with its relevance, surely an important indicator that ingredients of a desensitizing injection should not be mixed according to the number and size of positives recorded on a skin test chart.

R.H. CHAMPION

Host-Parasite Relationships in Systemic Mycoses. Part I: Methodology, Pathology and Immunology. Part II: Specific Diseases and Therapy. Proceedings of the 21st Annual OHOLO Biological Conference, Ma'alot, Israel, 1976. Edited by A.M. BEEMER, A. BEN-DAVID, M.A. KLINGBERG and E.S. KUTTIN, Basel; Karger. Pp 178. SFr 78
Contributions to Microbiology and Immunology volumes 3 and 4, Basel, 1977.

The 1976 Ma'alot Symposium on systemic mycoses was a gathering of some of the foremost experts in the world of medical mycology with a corresponding variety of interests. The purpose was to underline some of the more important recent advances in fungal disease as they appeared to each contributor. This makes a pleasant change from the single minded approach of most textbooks, although it has the disadvantage that certain sections are covered in meticulous detail, e.g. antigens of *Histoplasma*, while others are given a more superficial airing. The title ‘Host-Parasite Relationships in Systemic Mycoses’ is perhaps a little misleading as more emphasis is placed on reviews of disease processes, diagnostic procedures and therapy.

In the first part (Part I: Methodology, Pathology and Immunology) there are a number of interesting sections including the influence of mycotoxins on fetal development and an excellent review of the recent work on spherulin vaccine in coccidioidomycosis. There is also a helpful and extremely practical guide to the diagnosis of systemic mycoses which will be of great use to clinicians. The second part (Part II: Specific Diseases and Therapy) is largely concerned with disease processes and treatment. Included here are the ocular mycoses and systemic candidiasis as well as histoplasmosis and certain others. It is

here that the interests of the writer can lead the reader away from some of the more intriguing recent advances. For instance, some of the recent work on mechanisms of immunological resistance to human cryptococcosis is barely mentioned. However, there is much new here including a chapter on the use of fungal vaccines, which probably have a part to play in the management of some chronic mycoses. In this section there is also a review of the current status of chemotherapeutic agents for the systemic mycoses which contains basic and practical advice on the use of certain antifungal drugs.

Of particular interest to the dermatologist is an excellent chapter on current concepts of mycetoma, with a recent classification of the species involved, and the sections on chemotherapy. However, it is unfortunate that neither sporotrichosis nor chromomycosis are mentioned in this symposium. The chapter on pathogenesis of mucocutaneous mycoses contains an interesting review of mechanisms. Unfortunately the clinical classification at the end of this chapter will not be familiar to many readers, for instance chronic mucocutaneous candidiasis is cited as an example of 'primarily chronic polyphlegmatic granulomatous candida folliculitis'.

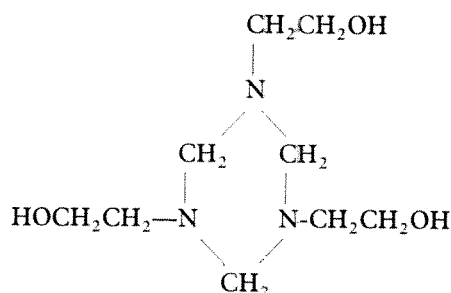
Generally speaking this is an excellent production which comes in two paperback volumes. The indexing is accurate and the diagrams are generally well presented. There is much information here for anyone with an interest in medical mycology whether dermatologist, immunologist or physician, and it should find a place in the well stocked medical library. Such a symposium has an important place in medical mycology where many of the concepts and treatments may change in a short time, but years may elapse between editions of the standard textbooks.

R.HAY

Review Article

Is Grotan BK a contact sensitizer?

Grotan BK is an industrial bactericide, widely used in the United Kingdom, continental Europe and the United States. It is marketed for addition to water-miscible, metal-working fluids of the oil-in-water emulsion ('soluble oil') and synthetic types. Water-miscible fluids provide cooling and lubrication for metal-working processes such as cutting and grinding. Bacterial growth in these fluids, which occurs particularly at oil-water interfaces, can cause problems of foul odour, metal corrosion and emulsion breakdown (Miller & Litsky, 1976). The active ingredient of Grotan BK is hexahydro-1,3,5,-tris(2-hydroxyethyl)-s-triazine:



Its recommended usage concentration in a metal-working fluid is 0.15%. Grotan BK is a member of a family of bacteriocidal chemicals, the triazines, which are also used to prevent spoilage of some cosmetics and hair-shampoos. Their precise mode of action is not known. The triazine molecule liberates formaldehyde under certain conditions but this is said by the manufacturers not to explain the anti-bacterial effect of Grotan BK.

Men and women working on machine tools can have heavy skin exposure to water-miscible fluids and contact dermatitis is commonly described as a result (Samitz, 1974). In 1971 it was estimated that there were approximately one million machine tools of all kinds in the United Kingdom and that some 80% of machining operations used water-miscible fluids (Cookson, 1971). Whether or not a bactericide widely used in such fluids is a contact sensitizer is therefore a question of considerable dermatological importance.

Opinion in the literature has been divided. Reports of sensitization were published in Germany in 1964 and were based on the results of patch testing 2156 workers in contact with Grotan BK (Borelli & Döngemann, 1964; Döngemann, Borelli & Reber, 1964). Clinically, 347 (16.1%) of these workers were diagnosed as having contact dermatitis. Patch testing was initially carried out in all 2156 workers with a series of possible allergens, including a mixture of Grotan BK with another triazine bactericide (both 1.0% in water). One hundred and forty-four subjects gave strongly positive reactions to this mix and 124 of these had previously been diagnosed clinically as having contact dermatitis. All 144 were then patch tested with Grotan BK and the other bactericide separately and at varying concentrations. Grotan BK at a patch test concentration of 0.5% gave 22 positive reactions and at a concentration of 1.0% gave 38 positive reactions. This represented a sensitization rate to Grotan BK

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of 1.0–1.8% in the total exposed population. The large majority of workers with positive patch test reactions to Grotan BK also had contact dermatitis on clinical diagnosis.

In the same year Rietschel (1964) reported a series of patch tests in workers in contact with Grotan BK in grinding fluid. He divided one group of 152 grinders into 50 with eczematous dermatitis and 102 without and patch tested them all with Grotan BK 0.15% in water. He found six positive reactors in each group, representing 12% of those with dermatitis and 6% of those without. In his discussion of these results the author suggested that Grotan BK was a weak sensitizer, more able to sensitize a skin already impaired by dermatitis than a skin that was still intact.

Another large series of patch tests was reported the next year by Schneider *et al.* (1965) with entirely different results from those of previous authors. Schneider reported patch tests carried out in a total of over 200 workers in contact with Grotan BK in soluble oil emulsions. Some 18% of these workers were described as having eczematous, erythematous or clinically allergic skin rashes. Patch test concentrations of 0.1–1.0% in water gave negative results in all subjects. The observed contact dermatitis was subsequently ascribed to a temporary change in the composition of the oil base of the emulsions.

Recently Keczkcs & Brown (1976) reopened the question with an account of patch testing 19 workers with dermatitis from a total of 300 in contact with Grotan BK. Positive reactions with a 0.2% aqueous solution of Grotan BK were obtained in four patients, 1.3% of the total exposed group.

In summary, patch tests in occupational groups exposed to Grotan BK have demonstrated sensitization rates ranging from zero (Schneider, 1965) to 8% (Rietschel, 1964). It appears, therefore, that Grotan BK may sensitize under some circumstances and not under others. Assistance in the further identification of circumstances favouring Grotan BK sensitization has come from a French report (Hesbert *et al.*, 1977). The results of guinea-pig maximization studies, carried out in the manner described by Magnusson & Kligman (1970), were here recorded. At concentrations of Grotan BK from 0.1% to 2.0% in vaseline sensitization did not occur, but a 4.0% concentration sensitized 10% of 20 guinea-pigs. Intradermal injection of 1.0% Grotan BK in olive oil sensitized 100% of animals. Grotan BK was thus shown, in the guinea-pig, to be a mild sensitizer when applied topically but a consistent sensitizer if injected intradermally. These results suggest that, in the guinea-pig, Grotan BK is potentially a stronger sensitizer than its poor ability to penetrate the epidermis allows it to be, when topically applied. Because eczema increases epidermal permeability, Rietschel's (1964) hypothesis that pre-existing eczematous dermatitis in grinders increased the likelihood of their sensitization by Grotan BK has thus received some experimental support. Studies of the percutaneous absorption of Grotan BK should enable this explanation to be further evaluated.

All patients with apparent contact dermatitis, in whom contact with Grotan BK is established, should be patch tested with a 1.0% solution of Grotan BK in water. Patch tests to date in a series of over 30 such patients at St John's Hospital for Diseases of the Skin have all been negative. Factors such as low usage concentration and, perhaps, poor epidermal penetrability seem to be capable of protecting machine tool operatives from the sensitization potential that Grotan BK has been shown to have in the guinea-pig. It appears, however, that circumstances may occasionally combine to produce the right conditions for sensitization to occur. The demonstration of a positive patch test reaction to Grotan BK should therefore be followed by a detailed enquiry as to what these predisposing circumstances might have been.

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REFERENCES

- BORELLI, S. & DÜNGEMANN, H. (1964) Aktuelle Kontaktekzem-Ursachen in der Metallindustrie. *Berufsdermatosen*, **12**, 1.
- COOKSON, J.O. (1971) Machine tool design and use in relation to cutting fluids. *Annals of Occupational Hygiene*, **14**, 181.
- DÜNGEMANN, H., BORELLI, S. & REBER, E. (1964) Kontaktallergien gegen eine Gruppe neuer Desinfektionsmittel. *Medizinische Klinik*, **59**, 170.
- HESBERT, A., DARRIGRAND, M.C., LEMONNIER, M. & CAVELIER, C. (1977) Study of bacterial contamination of cutting fluids. Efficiency of preservatives on micro-organisms. *Archives des Maladies Professionnelles*, **38**, 569.
- KECZKES, K. & BROWN, P.M. (1976) Hexahydro 1,3,5, tris(2-hydroxyethyl) triazine, a new bacteriocidal agent as a cause of allergic contact dermatitis. *Contact Dermatitis*, **2**, 92.
- MAGNUSSON, B. & KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis In The Guinea-Pig*. Charles C. Thomas, Springfield, Ill.
- MILLER, B.M. & LITSKY, W. (1976) *Industrial Microbiology*. pp. 384-411. McGraw-Hill, New York.
- RIETSCHEL, E. (1964) Erfahrungen aus der werksärztlichen Praxis über Hautschaden bei Nassschleifern. *Berufsdermatosen*, **12**, 284.
- SAMITZ, M.H. (1974) Effect of metal working fluids on the skin. *Progress in Dermatology*, **8**, 11.
- SCHNEIDER, W., HUBER, M., KWOCZEK, J.J., POPP, W., SCHMITZ, R. & TRONNIER, H. (1965) Weitere Untersuchungen zur Frage der Hautverträglichkeit hochverdünnter Kühlmittel. *Berufsdermatosen*, **13**, 65.

News and Notices

Annual Meeting of ESDR, 11-14 June 1979

The 1979 Annual Meeting of the European Society for Dermatological Research (ESDR) will be held jointly with the Society for Investigative Dermatology (SID). It will take place in the RAI Congresscentrum, Amsterdam, 11-14 June, 1979.

Attendance at the scientific sessions will be open to all members, contributors and guests. Non-members who wish to present a paper may submit an abstract. They will be invited to attend the meeting as either contributors or guests.

Abstracts should be presented on the official form. The deadline for submission of abstracts to the Joint Meeting through the ESDR will be 15 December 1978. The deadline for submission of abstracts through the SID will be 15 January 1979. However, all abstracts will be considered simultaneously by a Joint Programme Committee made up of both the ESDR and the SID.

Abstract forms and information about the meeting can be obtained from M.W. Greaves, ESDR Secretary, Institute of Dermatology, Homerton Grove, London E9 6BX, U.K.

European Society for Dermatological Research Poster Award

The ESDR poster award for the best poster presentation at the annual ESDR meeting has been kindly offered by Max Ritter, Pharmaceutical Specialities, Zurich/CH. The winners for 1978 are P. Fritsch, F. Gschnait, G. Kaaserer, W. Brenner, H. Kraus, H. Honigsmann and K. Wolff, Departments of Dermatology, University of Innsbruck and University of Vienna, for the poster 'Animal model for PUVA: partial suppression of proliferative stimulus induced by stripping'.

APPOINTMENTS

University of Glasgow

The University of Glasgow has appointed Rona McLeod MacKie to the Chair of Dermatology in the University. Professor MacKie took up her post, the first woman ever to hold an established Chair in the University, on 1 April 1978. She has been an Honorary Clinical Lecturer in the University Department of Dermatology and a Consultant Dermatologist with the Greater Glasgow Health Board. Dr MacKie succeeds the late Professor John A. Milne, who died in July 1977.

A native of Dundee, Dr MacKie attended schools in Aberdeen and London and, finally, Laurel Bank School in Glasgow. She entered Glasgow University as an Oliphant Entrance Bursar and graduated M.B., Ch.B. in 1963. She was awarded the degree of M.D. with Commendation by the University of Glasgow in 1970 and gained membership of the Royal College of Physicians in 1971.

Dr MacKie spent periods of residency in the Western and Victoria Infirmarys in Glasgow, and until 1972 worked in the Department of Dermatology at the Western Infirmary. During this time she served a brief period as a Lecturer in the University's Dermatology Department. She had held her previous post since 1973, undertaking clinical and postgraduate teaching duties at the Western Infirmary, the Southern General Hospital and the Royal Hospital for Sick Children, all in Glasgow.

Dr MacKie's research interests lie in two inter-related fields of the study of malignant diseases of the skin. She has studied methods for the early clinical detection of these diseases and, in particular, of malignant melanoma and is also involved with research into the immunological aspects of the diseases, with a view to the possible use of immunological markers in their detection. As a member of the WHO Malignant Melanoma Study Group, Dr MacKie is the British Trials Officer for a long-term study of the relative benefits to the patient of chemotherapy and immunotherapy for malignant melanoma after surgical removal of the lymph nodes involved in the disease.

Consultant to the Royal Navy in Scotland

Dr N. Morley has been appointed Civil Consultant to the Royal Navy in Scotland. He did National Service in the navy and took part in the Suez Operation in 1956. Since leaving the navy he has been in the Royal Naval Reserve and was awarded the Reserve Decoration in 1969.

Dr Morley is Consultant Dermatologist at the Western Infirmary and Royal Hospital for Sick Children in Glasgow.

The Royal College of Physicians, London

The following dermatologists have recently been elected to the Fellowship of The Royal College of Physicians, London:

Dr J.L.Burton	Dr R.B.Fountain	Dr J.L.Verbov
Dr M.A.Cowan	Dr C.Hodgson	Dr J.E.White
Dr J.J.Cream	Dr G.M.Levine	
Dr R.P.R.Dawber	Dr J.A.Savin	

Progress in Cutaneous Immunopathology

A meeting, entitled 'Progress in Cutaneous Immunopathology', conducted under the auspices of the 'Institut National de la Santé et de la Recherche Médicale' will be held on 27-28 October 1978, in Lyon, France.

The Meeting will be conducted in English and French and will comprise four sections:

In vivo fixation and cytotoxicity of antibodies in skin diseases.

Immune complexes and complement in skin diseases.

Surface markers and antigens of the epidermal cells and cutaneous lymphoma cells.

Chemotaxis and cutaneous diseases.

For additional information, please write to: Professeur J.Thivolet, FRA INSERM No. 11 'Recherche Dermatologique et Immunologie,' Clinique Dermatologique, Hôpital Edouard Herriot, 69374 Lyon Cedex 2, France.

European Society for Dermatological Research Symposium: Vasculitis Department of Dermatology, University of Innsbruck, 16 and 17 February 1979.

This is the 4th of a series of clinically oriented symposia of the ESDR. The session will consist of papers of invited speakers, discussion periods and free communications. Anyone wishing to contribute or to attend should contact Professor K.Wolff, Department of Dermatology, University of Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria.

New York University School of Medicine. Sherlockian Dermatopathology. Subtle Clues to the Diagnosis of Skin Diseases 19-20 October 1978

21-22 October 1978. Workshop on 'Histologic Diagnosis of Inflammatory Skin Diseases'

The title of this symposium, Sherlockian Dermatopathology, was coined to suggest the possibility of applying to dermatopathology and general pathology the methods of deductive and analytic logic practised by Sherlock Holmes. The elusive criminals in dermatopathology are diseases of the skin. The faculty of skilful dermatopathologists here assembled will demonstrate how these cutaneous miscreants can be tracked down and exposed by utilizing Sherlockian principles, stratagems, and lenses of various magnifying power. Although the emphasis will be on histological clues to diagnosis by conventional

microscopy, clinical, electron-microscopical, and immunopathological aspects of inflammations, neoplasms, malformations, and deposits of abnormal materials in the skin will also be scrutinized.

On the Saturday and Sunday immediately following the symposium on Sherlockian Dermatopathology, Dr Ackerman will give an intensive course on Histologic Diagnosis of Inflammatory Skin Diseases in the form of lectures and demonstrations covering the nine patterns of inflammatory diseases in the skin. Preference for registration, which is limited to fifty, will be given to those who attend the Symposium on Sherlockian Dermatopathology.

Further information about these Courses may be obtained from Dr A.B. Ackerman, Dermatopathology Section, New York University Medical Center, 562 First Avenue, New York, N.Y. 10016, U.S.A.

Symposium of Cutaneous Tumours in Children

A Symposium of Cutaneous Tumours in Children will be held in Ghent on 17 November 1978. This will be followed on 18 November by a meeting of the Belgian Society for Dermatology and Syphilology. Further information may be obtained from Professor Dr J. De Bersaques, Dermatologie—Akademisch Ziekenhuis, De Pintelaan 135, B-9000 Ghent, Belgium.

International Society of Tropical Dermatology

An Interim-Congress Symposium of the International Society of Tropical Dermatology will be held in Israel on 2–11 November 1978. The Symposium will be conducted in English, and the Scientific Programme will include the following topics: Immunology, Leishmaniasis, Tropical Dermatoses and Free Communications.

Those interested in participating and/or submitting a paper should contact Professor Jacob Sheskin at 4 Bet Hakerem, Jerusalem, Israel.

Investigative Meeting British Association of Dermatologists 19 & 20 January 1979, London

This meeting will take place on the afternoon of 19 (Friday) and the whole of 20 (Saturday) January 1979. It will take place at St. George's Hospital Medical School, Tooting, London.

Please could abstracts be forwarded to: Dr. W.J. Cunliffe, Consultant Dermatologist, Department of Dermatology, The General Infirmary, Great George Street, Leeds LS1 3EX, by not later than 30 October 1978. (Abstracts not received by this date will not be considered.)

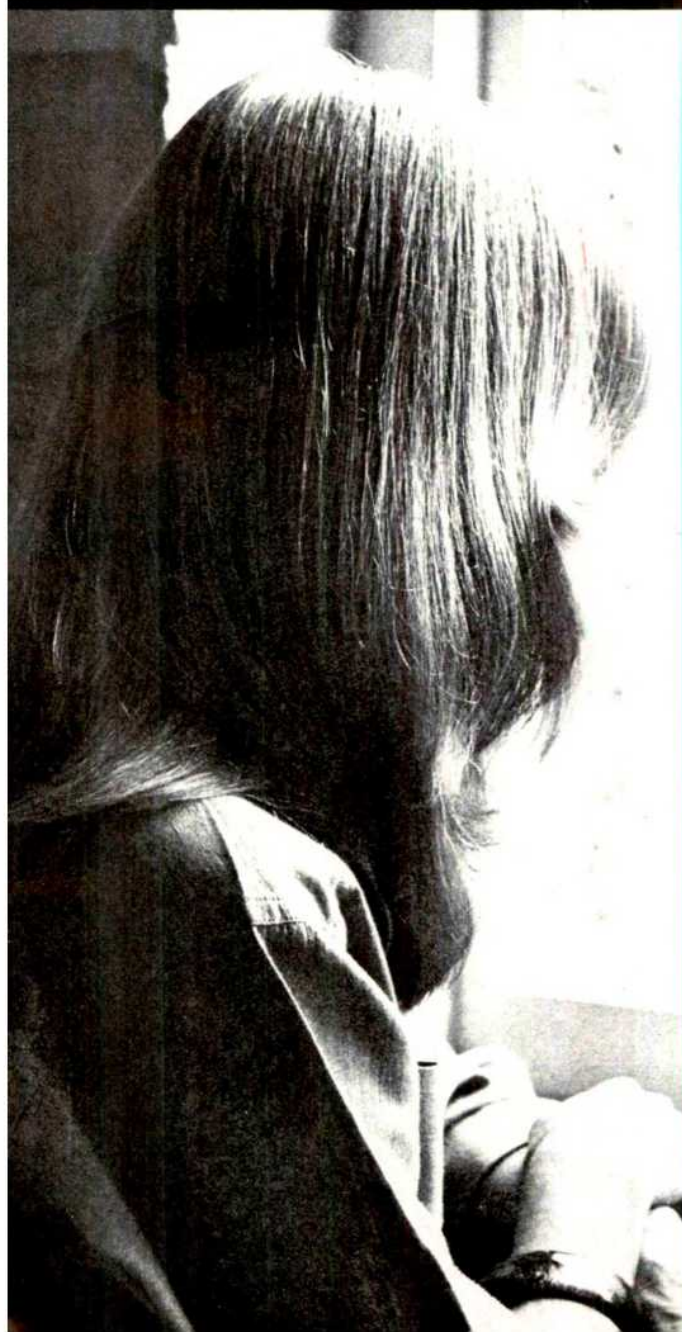
Seven copies of the abstract should be typed double-space on 8.5 × 11 in or 21 × 29.5 cm paper. The abstract should consist of *not more than 250 words* and should contain a statement of the purpose of the study, methods used and a summary of the results presented in sufficient detail to support the conclusions. Supplementary information, such as electron micrographs and tables, can be submitted at the discretion of the author for the use of the selection committee.

Accommodation, at a reasonable price, will be arranged.

There will be a subsidized meal on the evening of Friday, 19 January 1979.

Full details of the meeting will be circularized to members of the B.A.D. and the Investigative Group, later in the autumn. Others interested should contact Dr. W.J. Cunliffe. The meeting is open to clinical and non-clinical colleagues.

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INSECTS AND OTHER ARTHROPODS OF MEDICAL IMPORTANCE

edited by **K.G.V. Smith**, *Department of Entomology, British Museum (Natural History), London.*

Intended primarily for the identification of insects and other arthropods of importance in human medicine. Each of the sixteen contributors is a specialist in the taxonomy of the group concerned, with, in some cases, practical field experience of arthropod-borne diseases abroad. There is considerable information on the role of disease vectors and their biology, from the malaria-carrying mosquitos to the more passively involved house-flies; the venomous stinging and biting insects and arachnids are also included. All groups are treated on a world basis. Extensive bibliographies provide a stepping stone to the specialist chapters and a detailed subject index facilitate use of the book by public health officers, forensic biologists and others not quite as directly involved as the medical entomologist for whom the book is mainly intended.

This book is being published in conjunction with the British Museum (Natural History)

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DERMATOTOXICOLOGY AND PHARMACOLOGY

edited by **F.N. Marzulli**, *Food and Drug Administration,*
and **H.I. Maibach**, *San Francisco Medical Center.*

Represents a first attempt at consolidating recent developments in various aspects of skin research which are thought to be of interest and concern to researchers in dermatotoxicology. Three key subdisciplines are skin irritation, skin sensitization and skin penetration. Also included are skin metabolism, skin carcinogenesis, and pharmacologic aspects of skin and immunology. (Series: Advances in Modern Toxicology Vol.4)

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This new index is designed to permit the accurate and efficient retrieval of information on dermatological diagnoses, and has a much wider scope than the index issued by the World Health Organization. The coding system is based upon the International Classification of Disease, which is understood and accepted universally, and it is entirely compatible with the Ninth Revision due to be inaugurated in 1979. It is compact yet inclusive of many new disorders, and will prove its value in epidemiological studies, in medical research and in the planning of health services. The coding system is becoming widely adopted throughout the world and dermatologists will therefore find this index to be increasingly useful in their practice.

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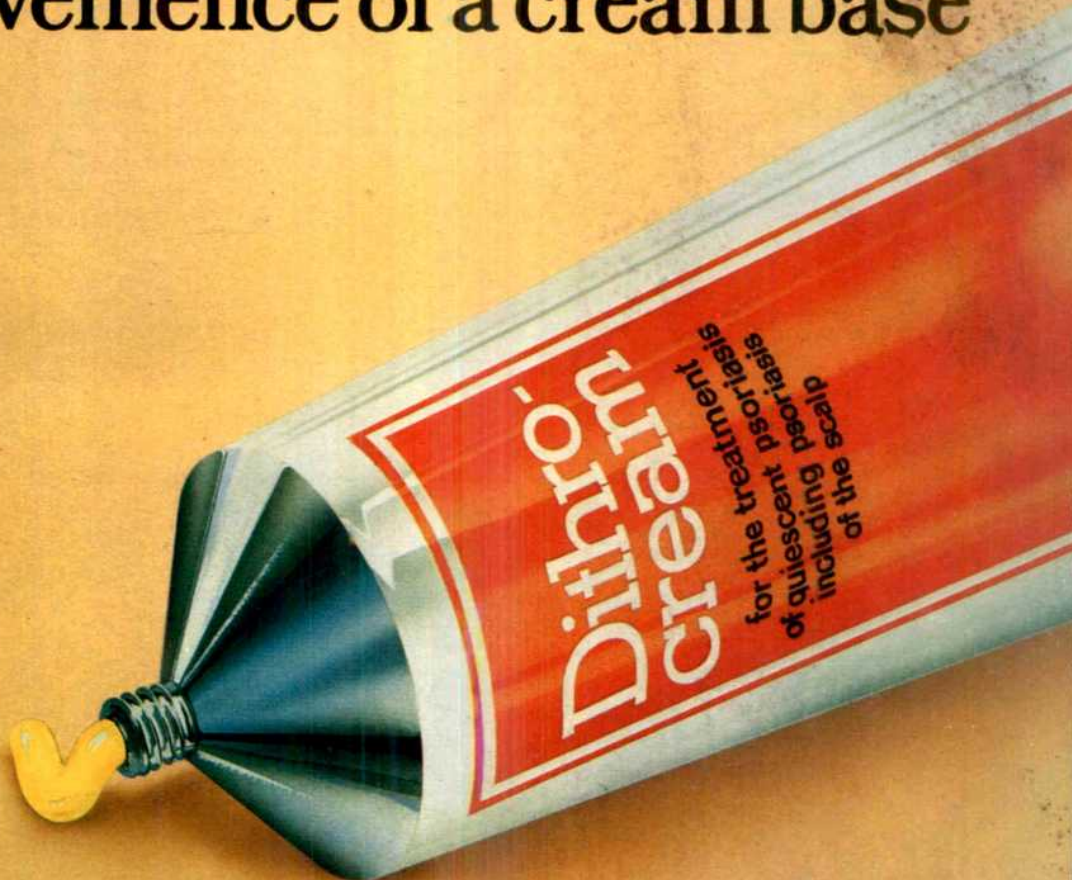
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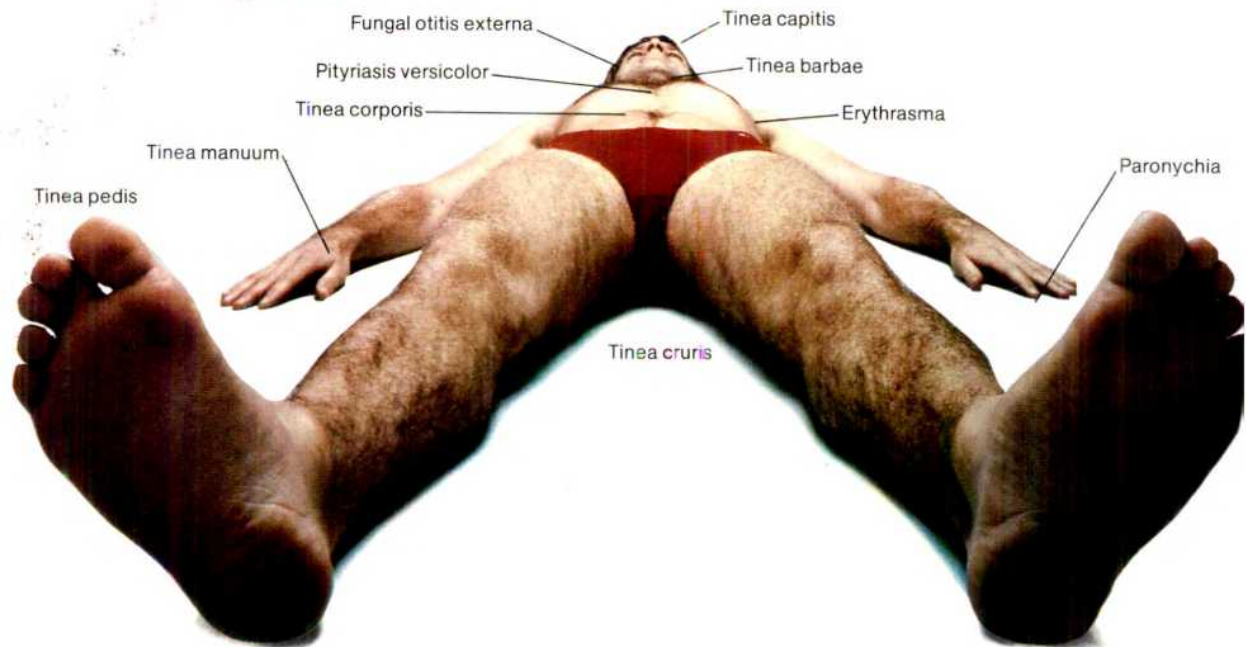
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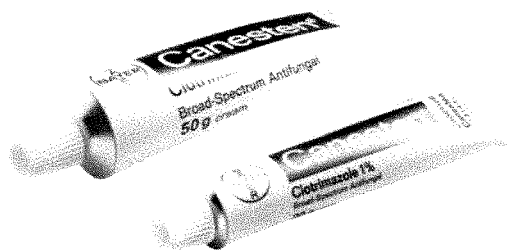
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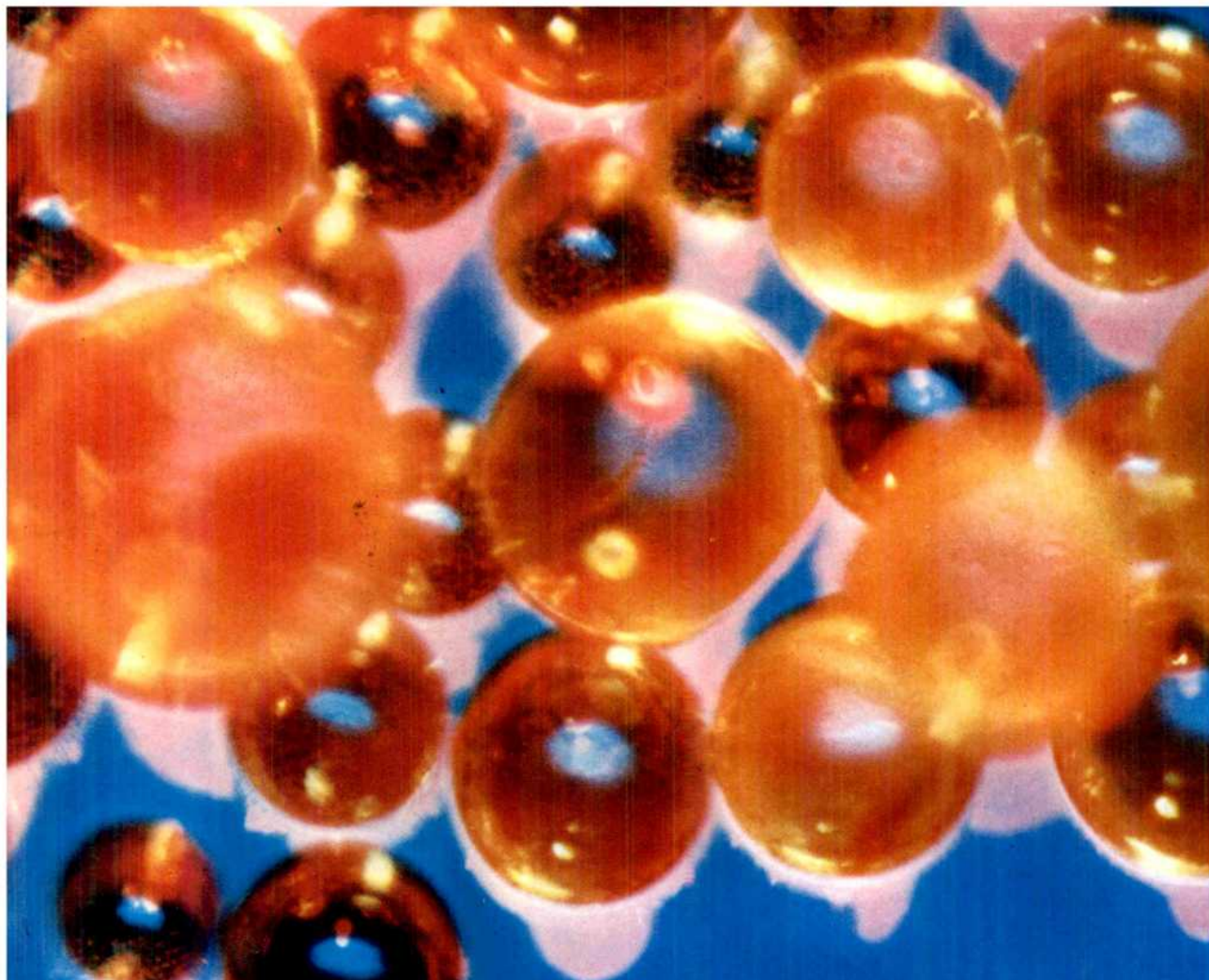


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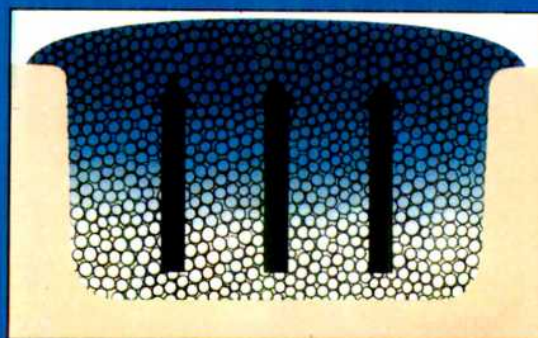
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Clinical Laboratory Investigations

Ultraviolet light inhibition of oriented fibrin formation in psoriasis

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SUMMARY

Radially-oriented fibrin crystallization was induced by incubation of blood from psoriatic patients with killed *Pseudomonas aeruginosa*. The phenomenon was inhibited by irradiation of the buffy coat in plasma with UV-B. It was not inhibited by UV-A unless trioxsalen had been added. Addition of UV-B-irradiated plasma to non-irradiated buffy coat also inhibits the fibrin formation.

An increased local fibrinoid deposition has been found in psoriatic skin areas and might have a role in the pathogenesis of psoriasis (Salo *et al.*, 1972; Fyrand & Husby, 1975). *In vitro* stellate fibrin microclots can be seen when blood of certain patients is incubated with bacterial products (Juhlin & Shelley, 1977). The centre of the focal microthrombi is usually a monocyte (Shelley & Juhlin, 1977). The phenomenon can be quantified by counting the number of stars formed per mm³. It is not seen in blood of healthy subjects but is common in patients with psoriasis, vasculitis, chronic urticaria, infected eczema and severe acne. In patients with psoriasis, the phenomenon tends to be strongly positive in the acute phase and can be weak or negative when the patients improve. As most of our psoriatic patients are treated with a bath containing trioxsalen followed by UV irradiation (Fischer & Alsins, 1976) and, since both blood and tissues are exposed to ultraviolet light, we decided to study the effect of UV light on fibrin formation *in vitro*.

METHODS

From patients with rather severe psoriasis, venous blood was drawn into 10 ml vacutainer glass tubes containing 144 units of heparin. One ml aliquots were mixed with endotoxin, bacteria or a chemical under study as recently described in detail (Juhlin & Shelley, 1977). After centrifugation in plastic tubes, the buffy coat and contiguous 2 cm of plasma were cut out, mixed and taken up into rectangular capillary tubes and sealed (Microslides, Vitro Dynamics, Rockway, N.J. 07866, Catalog No. 5005).

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The glass slides were mounted flat on a microscope slide and kept at 37°C for 24 h. The total numbers of crystalline star formations were counted using a phase interference (Leitz) microscope.

Chemicals added

(1) Killed bacterial cells, *Pseudomonas aeruginosa*, 10^9 /ml containing 0.4% phenol (Hollister-Stier Laboratories, Spokane, Washington, U.S.A.). The concentration of bacteria in blood was 10^4 /mm³.

(2) Lipopolysaccharide B (*S. enteritidis*, 3105-25, Difco), concentration 10^4 /mm³ blood.

(3) Trioxsalen, 5 mg was dissolved in 10 ml ethanol. Then one ml was mixed with 99 ml of sterile saline at 37°C and 0.01 ml was added to one ml of blood, making a final concentration of 50 ng/ml blood.

Irradiation

The narrow band lamps used for ultraviolet light irradiation and their characteristics have been described in detail elsewhere (Alsins *et al.*, 1976). The following irradiations were given:

(a) The 313 nm band, 0.1–0.5 J/cm².

(b) The 365 nm band, 0.5–10 J/cm².

The glass slides were placed on a 5 cm diameter irradiation tube with the capillaries towards the light. The light dose was measured with a power meter (Alsins *et al.*, 1976). For irradiation of the plasma portion of the centrifuged blood in plastic tubes, the lamp was turned at a right angle and the tubes irradiated in a vertical position.

RESULTS

Ultraviolet light B (300–320 nm) and trioxsalen + UV-A (340–380 nm) inhibited the oriented fibrin formation induced by pseudomonas in patients with psoriasis. UV-A irradiation without trioxsalen was without effect as was trioxsalen without irradiation. The doses and effects are summarized in Table 1.

When plasma from blood to which pseudomonas or lipopolysaccharide B had been added was re-mixed with the button of the buffy coat, the number of stars decreased. To study the effect of irradiated plasma on fibrin formation, the buffy coat and the plasma (1 cm) just above were therefore removed and re-mixed with an equal amount of plasma taken from the top of the centrifuged tube. If

TABLE 1. Effect of UV light and trioxsalen on fibrin formation

Treatment of blood in addition to pseudomonas					Number of fibrin stars in the tube	
Drug added	Dose (μ g/ml)	Type of light	Light dose (J/cm ²)	No. of patients	Range	Mean
—	—	—	—	20	25–230	89
+	—	UV-B	0.5–1	20	0	0
—	—	UV-B	0.1	6	0–45	11
—	—	UV-A	5–10	11	24–212	85
Trioxsalen	0.05	UV-A	10	10	0–33	10
Trioxsalen	0.05	UV-A	3–5	10	0–132	44
Trioxsalen	0.5	UV-A	5	10	0–68	20
Trioxsalen	0.05	—	—	11	15–214	80

the added plasma had been irradiated with UV-B (1 J/cm^2), the number of stars was reduced by over 50% in 11 of 15 patients, whereas plasma with $0.5 \text{ } \mu\text{g}$ trioxsalen per ml and irradiated with UV-A (5 J/cm^2) did not reduce the number of fibrin stars.

DISCUSSION

Evidence has recently been presented that the stellate fibrin formation is induced by monocytes (Shelley & Juhlin, 1977). The induction of focal fibrin formation by the monocyte may be related to its coagulant effect on exposure to *Escherichia coli* lipopolysaccharide in a one stage coagulation assay (Rivers *et al.*, 1975). This has been interpreted as evidence that the monocyte can generate a coagulant tissue factor. Further studies indicate that the lipid A moiety of the endotoxin is responsible (Rickles & Rock, 1976).

Parrish *et al.* (1977) in a preliminary report mentioned that monocytes and lymphocytes are sensitive to UV-A, B and C. The neutrophils might be less sensitive since we found them moving like non-irradiated cells when viewed under the microscope 2–4 h after irradiation. Swanbeck *et al.* (1975) described chromosomal aberrations in lymphocytes from patients treated with 8-methoxypsoralen when they were irradiated *in vitro* with UV-A (0.1 – 0.6 J/cm^2). Recently Krüger *et al.* (1978) found that when human lymphocytes were incubated with 8-methoxypsoralen and irradiated with UV-A, the mitogenic response to PHA was suppressed. The minimal dose giving inhibition was $0.1 \text{ } \mu\text{g/ml}$ 8-methoxypsoralen, which is close to that used by us.

We don't know if the microclots induced *in vitro* by bacterial products in blood from psoriatic patients also occurs *in vivo*. They indicate, however, that the patients have an abnormal capacity to form fibrin and this is in response to specific bacterial endotoxins. As a correlate we know from clinical experience that infections can precede eruptions of psoriasis. The number of fibrin microclots that can be induced *in vitro* is usually increased when the psoriasis becomes worse and can decrease upon healing (Juhlin, Valquist & Shelley, in preparation). Endotoxins have also been found to precipitate an altered form of fibrin called heparin-precipitable fraction (HPF), cryopofibrin, or soluble fibrin monomer complex (Fyrand & Husby for ref., 1975). HPF was, however, detected in only 6% of the patients with psoriasis vulgaris as compared with 48% of those with arthropathic psoriasis (Fyrand & Husby, 1975). A local precipitation of fibrin, fibrinogen or fibrin degradation products was described in both psoriatic and unaffected skin in 67 and 50% respectively of the patients (Fyrand & Husby, 1975). Salo *et al.* (1972) reported an increased accumulation of fibrin products in the dermo-epidermal junction and in the capillary walls of the papillae in 2 of 4 patients with guttate psoriasis after streptococcal tonsillitis and in 13 of 21 patients with 'unstable' psoriasis. A fibrin-like material in the dermis is not specific for psoriasis. In a different pattern it has also been described in cell-mediated hypersensitivity, lichen planus and dermatitis herpetiformis (Colvin *et al.*, 1973; Salo *et al.*, 1972; Mustakallio *et al.*, 1970).

One can always question if the patients form fibrin stars because they have widespread psoriatic skin lesions or whether they have skin lesions because of increased fibrin formation. As in many other inflammatory diseases, such as vasculitis and glomerulonephritis, fibrin seems to be a pathogen, although it sometimes can initiate a repair process (Ryan, 1976).

If we assume that microclots or an increased fibrin formation are involved in the skin lesions, can light reaching the blood during treatment be of such a magnitude that it affects fibrin formation? The doses of light for the treatment of psoriasis are of the same magnitude as those used in the *in vitro* experiments. The thin glass in the capillary tubes (0.05 mm) would probably not stop more than 40% of the 313 nm light and would let through almost all of the 365 nm light. Since the 365 nm light can penetrate the skin to basal cell layers and the capillary bed, our experiments would suggest that it

would definitely inhibit fibrin formation in these areas in psoralen-treated patients. The 313 nm light probably acts largely in the epidermal area where 85–94% is absorbed. As it is about 50 times more active than the 365 nm light, it might still have some action on the blood cell responsible for stars, namely the monocyte.

An additional possible effect of UV irradiation could be the release in the epidermis of an inhibitor to fibrin formation. Thus, the PUVA treatment might affect tissue clotting *in vivo*. If it is absorbed into the blood, it might also explain the decrease of stars often found when the patients had been treated with UV-A and UV-B for some time. Our findings that irradiation of plasma alone with UV-B can form a fibrin-inhibiting factor would strengthen such a possibility for short UV light. However, as plasma with trioxsalen irradiated with UV-A did not inhibit fibrin formation, a direct effect on the monocytes seems here more plausible.

ACKNOWLEDGMENTS

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REFERENCES

- ALSINS, J., CLAESSON, S., FISCHER, T. & JUHLIN, L. (1975) Development of high intensity narrow-band lamps and studies of the irradiation effect on human skin. *Acta dermato-venereologica*, **55**, 261.
- COLVIN, R.B., JOHNSON, R.A., MIHM, M.C. JR & DVORAK, H.F. (1973) Role of clotting system in cell-mediated hypersensitivity. I. Fibrin deposition in cell-mediated hypersensitivity. *Journal of Experimental Medicine*, **138**, 686.
- FISCHER, T. & ALSINS, A. (1976) Treatment of psoriasis with tri-oxsalen baths and dysprosium lamps. *Acta dermato-venereologica*, **56**, 383.
- FYRAND, O. & HUSBY, G. (1975) Deposition of fibrinogen (FR-antigen) in skin diseases. I. Psoriasis vulgaris and psoriasis arthropathica. *Acta dermato-venereologica*, **55**, 175.
- JUHLIN, L. & SHELLEY, W.B. (1977) Oriented fibrin crystallization: a phenomenon of hypersensitivity to bacteria in psoriasis, vasculitis and other dermatosis. *British Journal of Dermatology*, **96**, 577.
- JUHLIN, L., VAHLQVIST, C. & SHELLEY, W.B. (1978) In preparation.
- KRÜGER, J.P., CHRISTOPHERS, E. & SCHLAAK, M. (1978) Dose-effects of 8-methoxypsoralen and UVA in cultured human lymphocytes. *British Journal of Dermatology*, **98**, 141.
- MUSTAKALLIO, K.K., BLOMQVIST, K. & LAIHO, K. (1970) Papillary deposition of fibrin, a characteristic of initial lesions of dermatitis herpetiformis. *Annals of Clinical Research*, **2**, 13.
- PARRISH, J.A., MORISON, W.L., ANDERSON, R.R. & BLOCH, K.J. (1978) Sensitivity of mononuclear cells to UV radiation. *Journal of Investigative Dermatology*, **50**, 218.
- RICKLES, F.R. & RICK, P.D. (1976) Structural features of *S. typhimurium* lipopolysaccharide (LPS) required for activation of monocyte tissue factor. *Federal Proceedings*, **35**, 804.
- RIVERS, R.P.A., HATHAWAY, W.E. & WESTON, W.L. (1975) The endotoxin-induced coagulant activity of human monocytes. *British Journal of Haematology*, **30**, 311.
- RYAN, T.J. (1976) Microvascular injury. *Major Problems in Dermatology*, vol. 7. Saunders, London.
- SALO, O.P., TALLBERG, TH. & MUSTAKALLIO, K.K. (1972) Demonstration of fibrin in skin diseases. I. Lichen ruber planus and lupus erythematosus. *Acta dermato-venereologica*, **52**, 291.
- SALO, O.P., KOUSSA, M., MUSTAKALLIO, K.K. & LASSUS, A. (1972) Demonstration of fibrin in skin diseases. II. Psoriasis. *Acta dermato-venereologica*, **52**, 295.
- SHELLEY, W.B. & JUHLIN, L. (1977) Induction of fibrin thrombi by monocytes. *Nature*, **270**, 343.
- SWANBECK, G., THYRESSON-HÖK, M., BREDBERG, A. & LAMBERT, B. (1975) Treatment of psoriasis with oral psoralens and longwave ultraviolet light. *Acta dermato-venereologica*, **55**, 367.

Mouse screening test for evaluating protection to longwave ultraviolet radiation

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SUMMARY

Albino mice photosensitized to psoralens were used for evaluating a sun-barrier preparation for use against longwave ultraviolet radiation. The difference in thickness of the treated and control ears at various times after irradiation was used as a measure of photoprotection.

Light protection in the UVA range (320–400 nm) is of importance, not only for preventive therapy of photodermatoses, but also in the avoidance of side effects in phototherapy, e.g. in PUVA treatment. Normally investigation of the photoprotective potency of a sun-barrier preparation might be studied initially on animals; humans are tested only later. Suitable methods on humans have been described by Maibach, Sams & Epstein (1967) and Dahlen *et al.* (1970). Using the mouse method of Sawa (1968) we have developed a screening method for evaluating the protective properties of topically applied sunscreens using the ear. One ear is treated, the other untreated; thus the animal acts as its own control. As we wished to study photoprotection to UVA, the mice were photosensitized with psoralen.

MATERIALS AND METHODS

Albino mice of both sexes, weighing approximately 30 g, were sensitized to UVA radiation by 0.2 ml subcutaneous 70% alcoholic 5-MOP and 8-MOP each 0.25%. The irradiation was started 30 min after the injection with a fluorescent lamp, LS 40 W UVA 70 (VEB Narva GDR-923 Brand-Erbisdorf). This lamp has a continuous spectrum between 290 and 400 nm, maximal at about 345 nm. The output of the lamp in the UVA spectrum, 1 m away, is 80 $\mu\text{W}/\text{cm}^2$. The animals, immobilized during irradiation, which lasted 5 h, were 5 cm away from the lamp, and wavelengths shorter than 320 nm were restricted by 4 mm-thick window glass.

Sixty animals, photosensitized and irradiated as above, but without any sunbarrier preparation on the ears, were used to test the validity of the method. The thickness of the ears was determined with a special instrument (Fig. 1); measurements were done before, and 24, 48 and 72 h after irradiation. The

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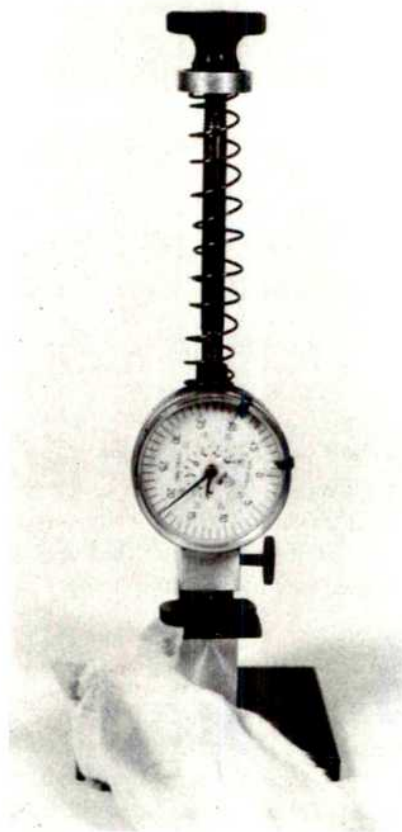


FIGURE 1. Instrument for measuring ear thickness of mice; a displacement dial gauge, slightly modified; ear placed between two platens to spread compression.

thickness of the ears was determined using a dial gauge for measuring displacement. The instrument was modified by adding a counterbalance spring as so to reduce pressure on the ear. The gauge very lightly compressed the skin of the ear between two platens which spread the load on the ear. The compression gives a slight inaccuracy to the absolute measurement of thickness but reproducibility of relative thickness in the oedematous ear was estimated to be better than $\pm 10\%$. Six days after irradiation, the ears were also examined for naked eye changes.

Another 36 mice, each photosensitized as above, were used for assessing the sunbarrier. To each of these animals, 6.0 mg of sunbarrier preparation cream (Contralum, Hermal-Chemie, FGR-Reinbeck) was applied to one ear; the active ingredient is a benzophenone. To the other ear a water-in-oil emulsion of wool fat alcohols 6.0 mg was applied as a control. The thickness of the cream to each ear was estimated to be approximately 30 μm .

RESULTS

The thickness of the normal ear in our mice lies between 110 and 220 μm . In the studies of the 60 photosensitized control mice, with no sunbarrier preparation, no significant difference in the thickness

TABLE 1. Effects of irradiation on mouse ear thickness (μm) mean and range

	Before irradiation	After irradiation		
		24 h	48 h	72 h
Ears pretreated with sunbarrier preparation	130 (110–150)	150 (130–160)	220 (130–400)	275 (150–470)
Control ears	130 (110–150)	155 (130–180)	285 (130–470)	335 (270–490)

of the ears was found between the two sides at 24, 48 and 72 h after irradiation ($P > 0.1$); the mean values for thickness (with ranges) at these times were respectively 191 (150–240), 367 (360–450) and 475 (350–550) μm .

Of the 36 mice where the sunbarrier preparation was applied to one of the ears, 4 died within 2 days of irradiation, but we have complete data from 32 pairs of ears. Table 1 shows the course of the change of the thickness of the ears during the experiment and compares the ears pretreated with those with the active sunbarrier preparation. Because of the relatively small number of animals and uncertainty as to data being normally distributed, we used the Wilcoxon test for paired values (Weber, 1972) as the statistical method for assessment. There was a significantly increased thickness on control sides 48 and 72 h after irradiation ($P < 0.01$). This difference in ear thickness did not occur at 24 h. Six days after irradiation, in the 29 surviving animals, necrosis was observed in 18 control ears, but in only 5 treated ears. Furthermore on visual inspection in 27 individual mice, the sunbarrier treated ear showed distinctly less thickening than the control ear.

DISCUSSION

Our method can be quantified and is suitable for dose-response and time-course determinations; also it is more economical of animals than the mouse tail method of Ljunggren & Möller (1976), for each animal acts as its own control. The method was chosen for evaluating sun-barrier preparations in the UVA spectrum, but it is also suitable for prospective determination of potential photosensitizers (Ison & Blank, 1967; Sawa, 1968; Barth, 1976) and for studies of the mode of action of chemicals already established as photosensitizing (Ljunggren & Möller, 1976, 1977). The subcutaneous injection of the photosensitizer allows us to use psoralens which are not water soluble. It was found that some care is necessary to ensure that the distribution of cream on the front and back of the ears is equal. A suitable instrument for measuring ear thickness, ensuring reproducible results, is of course essential. In view of these advantages, we can recommend this method as a screening test and for other investigations in the evaluation of photoprotective substances.

REFERENCES

- BARTH, J. (1976) Untersuchungen zur prospektiven Erfassung fotosensibilisierender Eigenschaften von Pharmaka und Industriesubstanzen. Med. Diss., Leipzig.
- DAHLEN, R.F., SHAPIRO, S.J., BERRY, C.Z.G. & SCHREIBER, M.M. (1970) A method for evaluating sunscreen protection from longwave ultraviolet. *Journal of Investigative Dermatology*, **55**, 164.
- ISON, A. & BLANK, H. (1967) Testing drug phototoxicity in mice. *Journal of Investigative Dermatology*, **49**, 508.
- LJUNGGREN, B. & MÖLLER, H. (1976) Phototoxic reaction to chlorpromazine as studied with the quantitative mouse tail technique. *Acta dermato-venereologica*, **56**, 373.
- LJUNGGREN, B. & MÖLLER, H. (1977) Phenothiazine phototoxicity: An experimental study on chlorpromazine and related tricyclic drugs. *Acta dermato-venereologica*, **57**, 325.

- MAIBACH, H.J., SAMS, W.M., JR & EPSTEIN, J.H. (1967) Screening for drug toxicity by wavelengths greater than 3100 Å. *Archives of Dermatology*, **95**, 12.
- SAWA, T.R. (1968) Phototoxicity screening procedures. Quoted by: STOTT, C.W., STASSE, J., BONOMO, R. & CAMPBELL, A.H. (1970) *Journal of Investigative Dermatology*, **55**, 335.
- WEBER, E. (1972) *Grundriss der biologischen Statistik*. Fischer-Verlag, Jena.

A comparison of the anatomical uniformity of irradiation in two different photochemotherapy units

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SUMMARY

Measurements have been made of the anatomical distribution of ultraviolet radiation resulting from the irradiation of an inanimate manikin in a PUVA 6000 unit and a PUVA 4000 unit. The dosimeter used was phenothiazine film. The results indicated a very similar pattern of relative UV dose on the surface of the manikin from both units.

It is customary in publications on the treatment of psoriasis by photochemotherapy (PUVA) for authors to quote the exposure dose of ultraviolet radiation received by the patient. Although it is inappropriate to specify a single UV dose due to the fact that topology of a human subject does not permit uniform irradiation, it is still important that the variation of UV dose over the anatomy is minimized. To this end we have studied the spatial variation of UV dose on an inanimate manikin resulting from irradiation in two commonly-used, commercial PUVA units.

MATERIALS AND METHOD

The UV dose was measured by use of the phenothiazine dosimeter described by Diffey *et al.* (1977). This dosimeter consists of a thin polymer film which incorporates phenothiazine as the chromophore. The film is cut up and made into film badges using 3 cm square cardboard photographic mounts. When exposed to ultraviolet radiation up to 400 nm wavelength, phenothiazine film exhibits a monotonic increase in optical absorbance, measured at 280 nm, with increase of ultraviolet dose.

The PUVA units used in this study were the PUVA 6000 and PUVA 4000 both manufactured by Waldmann in West Germany and containing GTE Sylvania high intensity lamps. The PUVA 6000 unit is a step-in cabin containing 62 linear sources. The PUVA 4000 unit contains 40 lamps mounted horizontally. The patient lies on a couch which is then wheeled under the unit.

Film badges were attached to 65 sites on the surface of an unclothed, male manikin (6 feet tall).

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The manikin was positioned in the PUVA 6000 unit in a posture closely approximating that of a human subject and given a UV dose of about 10 Jcm^{-2} as determined by the Waldmann PUVA meter. Since we are interested in only relative doses it was not necessary to estimate accurately the absolute dose. At the end of the exposure the change in optical absorbance on each film badge was measured and the UV dose calculated. The experiment was repeated in order to assess the reproducibility of positioning. It was found that the mean dose at a given site was within $\pm 10\%$ of the doses obtained on the two irradiations. The manikin was then positioned in the PUVA 4000 unit and the whole set of measurements repeated. Treatment with this unit necessitated irradiating the manikin in both a prone and a supine position with the same set of film badges in position.

RESULTS

Fig. 1 shows the relative distribution of UV dose on the manikin from the PUVA 6000 unit and Fig. 2 the same distribution resulting from irradiation on the PUVA 4000 unit. It may be seen that both units give roughly the same distribution of relative doses. A large fraction of the body surface area receives more than 70% of the maximum dose although areas such as the axillae and groin receive a

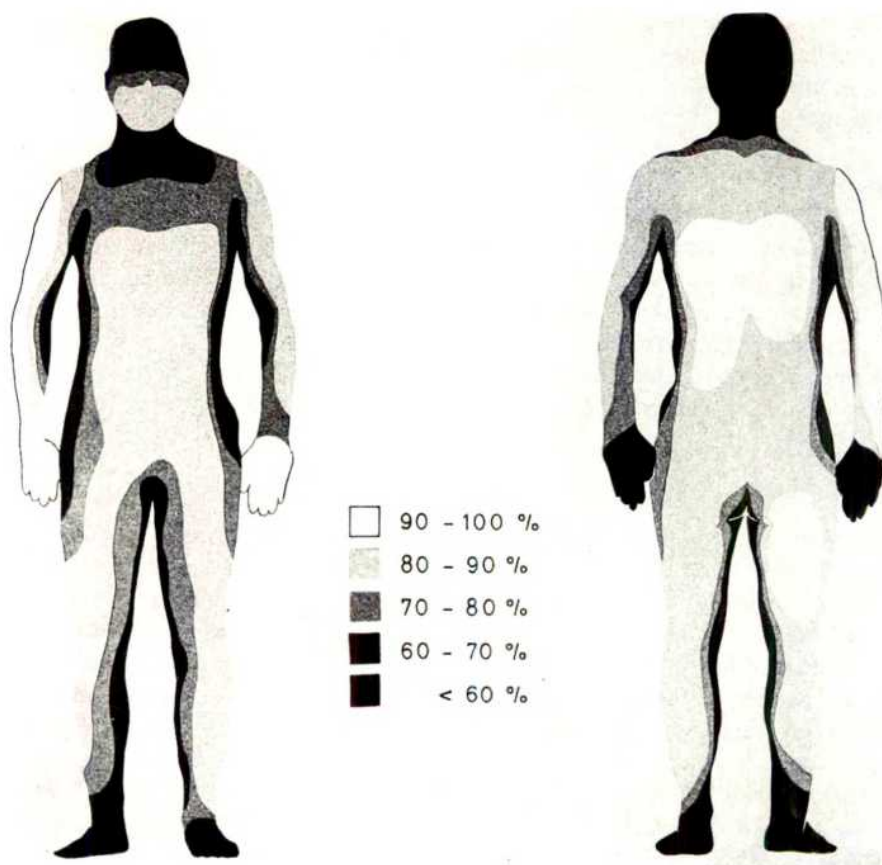


FIGURE 1. The relative distribution of UV dose on the surface of a manikin resulting from whole body irradiation in a PUVA 6000 unit.

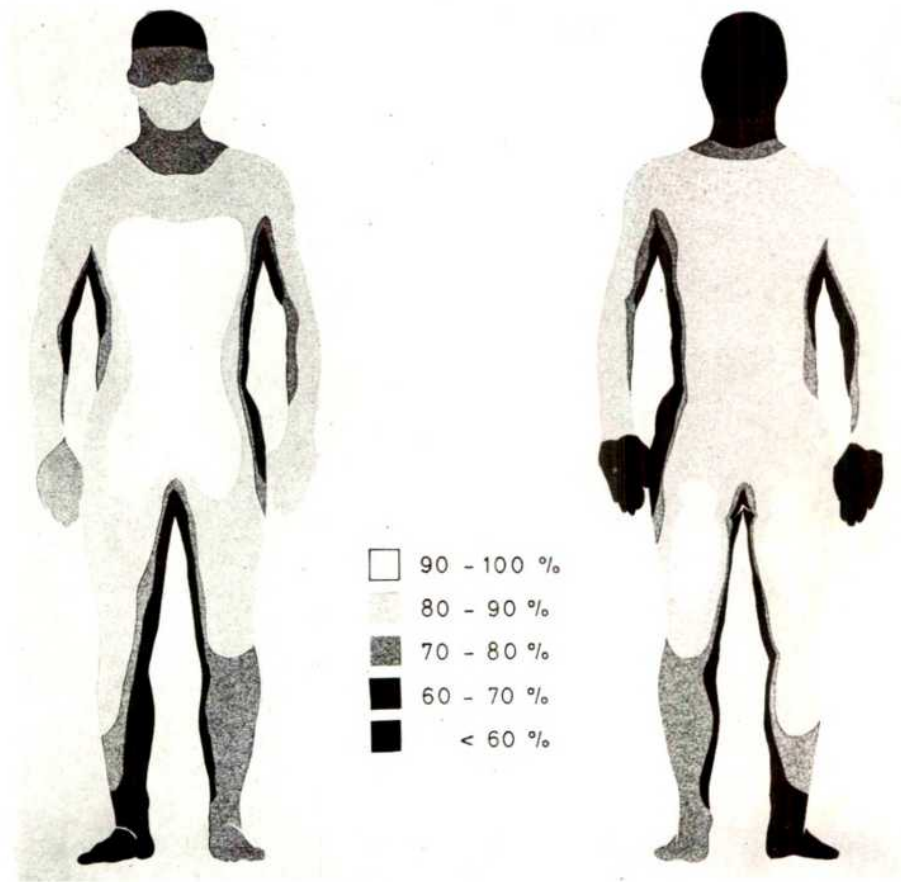


FIGURE 2. The relative distribution of UV dose on the surface of a manikin resulting from whole body irradiation in a PUVA 4000 unit.

smaller fraction, as expected. It is interesting to note in Fig. 1 that the right arm received about 10% more UV than the left arm, even though the manikin was positioned centrally in the cabin. This result was substantiated by measuring the UV irradiance within the cabin with the PUVA meter, where it was observed that the intensity was indeed higher from one side of the cabin than the other, presumably due to differential ageing of the lamps. This observation highlights the need for patients to shift their orientation occasionally during treatment to ensure as small a variation in spatial irradiation as possible.

REFERENCE

- DIFFEY, B.L., DAVIS, A., JOHNSON, M. & HARRINGTON, T.R. (1977) A dosimeter for long-wave ultraviolet radiation. *British Journal of Dermatology*, **97**, 127.

The effect of a single plucking at different times in the hair cycle on the growth of individual mouse vibrissae

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SUMMARY

Mouse vibrissae have been plucked at precise times throughout the hair cycle and the time of appearance of the new hair noted for each individual follicle. It was found that plucking a vibrissa at any time during the cycle resulted in the induction of a new hair and in no case did the follicle continue the same cycle. All the vibrissae grew at the normal rate of 1.0 mm/day for most of the growing period and whenever plucked during this period a new hair appeared after 8–11 days. When the growth rate fell to 0.6–0.7 mm/day at the end of the growing period plucking resulted in the appearance of a new hair after 7 days. When the growth rate fell to 0.1–0.3 mm/day a new hair appeared 5 days after plucking, thus the time of normal eruption was not altered.

When whiskers were 10–15 mm long and about half way through the active growth phase their follicles were surgically transected at one of three different levels: (1) just above the dermal papilla which left all or most of the differentiating matrix cells, (2) mid-follicle and (3) at the level of the sebaceous gland. In each case the distal part of the hair was then plucked. In five out of ten cases when the cut was made just above the dermal papilla a new hair erupted after a variable time 10–15 days. In all other cases the existing hair cycle was completed after a delay of a few days. This emphasizes the importance of the differentiated matrix cells in the maintenance of the hair cycle.

The growth of hair is known to be cyclic in all animals yet studied. Chase (1955) suggested that an inhibitor accumulates during the active phase (anagen) and becomes dispersed during the resting phase (telogen). In 1965, Bullough proposed the chalone hypothesis as an explanation of the cyclic activity in the hair follicle. However, the reason for the sudden initiation of anagen after a fixed period of telogen is still unknown.

Plucking a hair from a resting body hair follicle always causes the initiation of a new hair (Collins, 1918; David, 1934; Johnson & Ebling, 1964; Johnson, 1965; Silver *et al.*, 1967). This has been used as a tool to produce an even growth of hair in experimental animals.

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Johnson & Ebling (1964) and Johnson (1965) plucked rats' body hair at different stages during the cycle. The time of eruption of the new induced hair was variable.

The cycles of the vibrissae of mice are very regular and can easily be determined accurately. We therefore plucked mouse vibrissae on a single occasion at different times during the cycle and observed the effects.

In order to pluck the whisker without damaging the differentiating matrix cells, actively growing whiskers were surgically cut inside the follicle at three different levels and the existing hair was then plucked out. The results show clearly the importance of the presence of differentiated matrix cells in the initiation of a new cycle.

MATERIALS AND METHODS

Single plucking

Two female 40-day-old C₃H mice were used. The two mice were born on the same day. It has been found that whisker follicles are always at the same stage of activity at birth and if undisturbed this synchrony remains for most of their life. In this experiment vibrissal lengths were within a few mm of each other at the commencement of this investigation. The vibrissae used are E, F, G and H on both sides of the mice. They are the largest and most posterior column of mystacial vibrissal follicles in mice and rats. They all erupt at almost the same time having the same rate of growth and final length. Their rate of growth is 1.0 mm/day, their final length is about 30 mm. Each follicle has an identical 'twin' on the other side of the animal.

A detailed study of the arrangement, nomenclature, growth, final length and other characteristics of the mouse and rat vibrissae and their follicles have been described previously (Ibrahim & Wright, 1975). Using forceps and minimal force, whiskers were plucked at different intervals during the cycle. The growth of every plucked whisker was measured for a few days before plucking so that the rate of growth could be estimated. Two of the sixteen experimental whiskers (E right, mouse I and H left, mouse II) were used as controls.

Surgical transection

In order to remove parts of the whisker and at the same time to leave the differentiated matrix intact, six female 5-week-old Wistar derived rats were used. Under Nembutal anaesthesia (40 g/1 kg body weight) a vertical skin incision was made posterior to the mystacial whisker pad so as to expose the deep parts of the whisker follicle. Surgical cutting of the growing vibrissae was performed on the right side E, F, G, H follicles. The left side 'twin' of each vibrissae was used as its control. Twenty-two follicles were surgically cut at three different levels: eight were cut opposite the sebaceous gland, four at the mid-follicular level and ten were cut just above the tip of the dermal papilla.

The transection was carried out by making a small hole through the capsule at the level required, then very small fine scissors were passed through and a transverse cut was made through the hair canal and the surrounding layers of the outer root sheath. The distal part of the hair was gently pulled out.

OBSERVATIONS AND RESULTS

Single plucking

Seven actively growing whiskers ranging between 6.4 and 22.3 mm in length were plucked at different stages during the growth phase. They were all growing 1 mm/day on the day of plucking. Six other whiskers were plucked late in the cycle at a time when the growth rate was diminishing (three at 0.7, one at 0.6 and two at 0.3 mm/day). The fourteenth whisker was plucked while the follicle was resting on the day of plucking. Fig. 1 and Table 1 show the length of each whisker plucked, its rate of growth

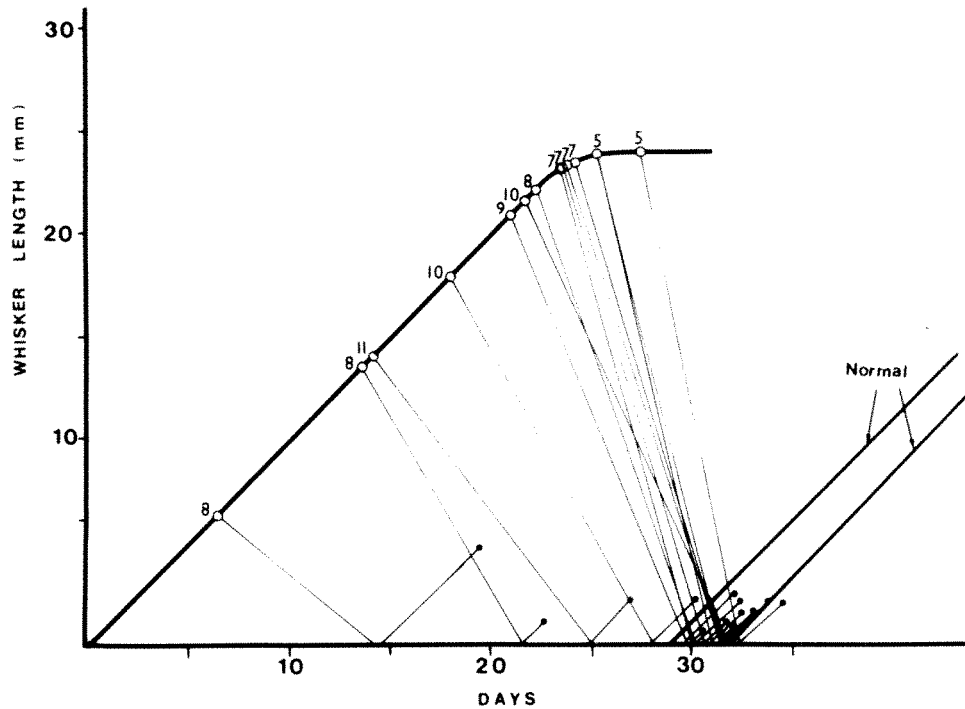


FIGURE 1. Shows the length and position on the growth curve of every plucked whisker and the day the new whisker was observed. By extending the growth curve of each new whisker backwards the day of its first appearance on the skin surface was estimated. The lines (with negative slope) are drawn to indicate the two cycles of each plucked whisker. The numbers beside each plucked whisker is the number of days between plucking and appearance of the new one. The two normal whiskers, E and H, were drawn for comparison. (○) Plucking time; (●) new whisker first observed.

at the plucking time and the number of days it took the new whisker to appear on the skin surface. Rate of growth of all the new whiskers was normal (1 mm/day). By knowing the rate of growth it was possible to find exactly the number of days elapsed between the plucking day and the day the new whisker appeared on the skin surface (Fig. 1). When the follicle was in the active stage at the time of plucking its whisker always took between 8 and 11 days to appear on the skin surface. This variation (of 3 days) was believed to be due to slightly different degrees of damage to the matrix during plucking. Figs 2a and b show the variable extent of tissue removed during plucking. When the follicle was entering its resting stage this period became shorter and shorter until a stage was reached when no change took place in the time of appearance of the new whisker. This suggested that the matrix was beginning to form the next hair and was uninfluenced by plucking the old one.

The growth of some of the new whiskers was followed for two generations. The rate of growth and all other cycle characteristics were normal and independent of the surrounding follicles.

Surgical transection

In the eight follicles in which whiskers were cut opposite the sebaceous gland, whiskers emerged after 3–5 days and in all cases they completed the same cycle. Whiskers cut at the mid-follicular level emerged after 4–6 days and also completed the same cycle. The hair was believed to be of the same cycle because the emerging hair had a cut tip, was of the same thickness as the plucked hair and grew for the expected length of time.

TABLE 1. The length and growth rate of each whisker on the day of plucking and the number of days elapsed between the day of plucking and the appearance of a new whisker on the skin surface

Whiskers at the time of plucking			No. of days between plucking day and appearance of the new whisker
Whisker	Length (mm)	Rate of growth (mm/day)	
IRF	6.4	1.0	9
IRG	13.6	1.0	8
IIRE	14.1	1.0	11
IRH	21	1.0	9
IIRF	18	1.0	10
ILH	21.7	1.0	10
IIRG	22.3	0.9	8
IIRH	22.2	0.7	7
IILF	23.2	0.7	7
ILF	24.1	0.7	7
ILE	23.6	0.6	7
IILE	23.8	0.3	5
IILG	23.7	0.3	5
ILG	17.3(a)	0.1	5

I and II=mice numbering code; R=right side; L=left side; (a)=tip was found broken at the first observation.

In five of the cases where the cut was made just above the tip of the dermal papilla whiskers emerged after 4–8 days and completed the same cycle. These whiskers had a blunt tip that was at first slightly finer than those of the plucked whiskers. The normal width was soon regained. This might be due to the fact that the cut was made at a part of the whisker which was soft and became distorted at the free tip.

In the other five follicles, new whiskers emerged after 10–15 days. This may have been due to the cut being slightly lower than intended so that the tip of the dermal papilla and upper matrix were damaged.

In the vibrissal follicles it seems that the differentiated cells of the matrix play a very important role in the cycle. Removal of this part of the matrix by plucking always resulted in an instant ending of the cycle. The matrix cells which were left behind will start dividing after a period of quiescence, leading to the initiation of a new cycle and the formation of a new hair. When the hair shaft was plucked (after surgical transection) above the differentiated matrix cells the follicle proceeded to finish the same cycle after a few days of delay. When the transection was made close to the tip of the dermal papilla the old cycle was continued in half the cases while a new cycle was initiated in the remainder. This demonstrates that the level near the tip of the dermal papilla is critical for new hair formation.

DISCUSSION

Collins (1918) and many more recent authors have showed that when a resting hair is plucked a new hair will start growing after a period of quiescence. Although this has been used as a tool to initiate activity in hair follicles (David, 1934; Johnson & Ebling, 1964; Silver & Chase, 1970; Jackson & Ebling, 1972), a full explanation of the mechanism is not known.

Butcher (1936), Ghadially (1957) and Silver, Chase & Arsenault (1967) believed that hair growth

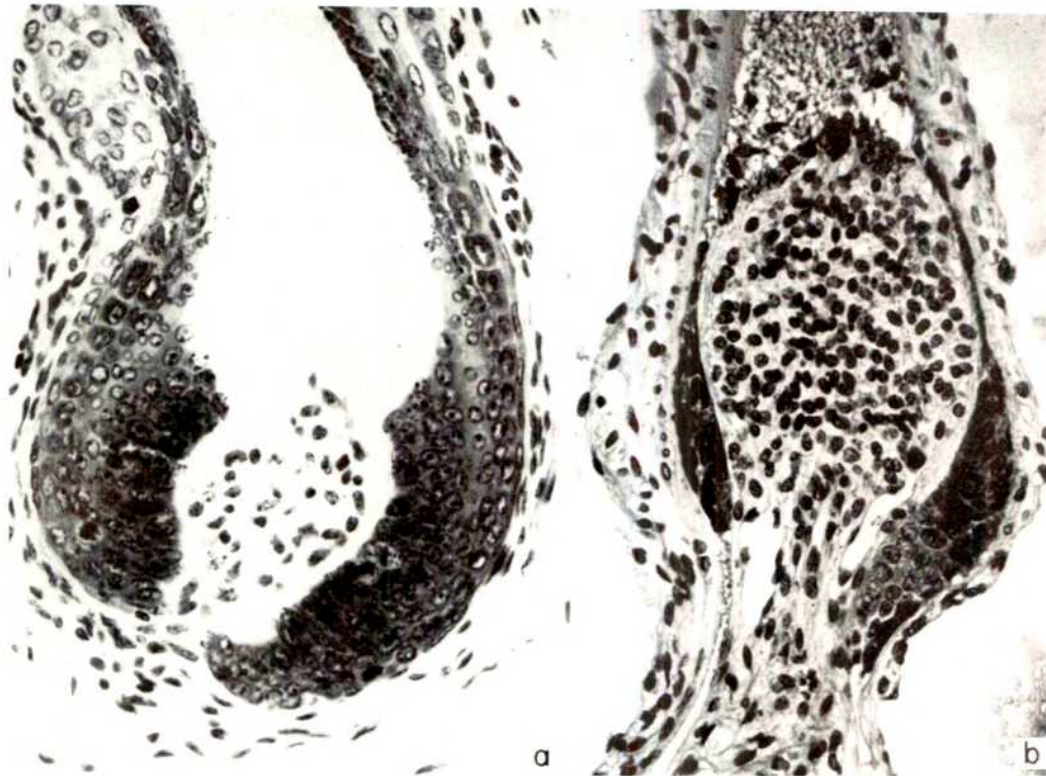


FIGURE 2. Longitudinal section through a plucked whisker follicle. (a) Most of the dividing matrix is left behind; (b) less matrix is left but dividing cells are still numerous (H & E, $\times 175$).

is controlled by means of intrinsic factors. The experiments described here show that the initiation of hair growth and control of the cycle in the vibrissal follicle are dependent on intrinsic factors. No follicle was influenced by any surrounding follicles.

Many theories have been put forward to explain the cyclic activity in the hair follicle. Chase (1955) assumed that an inhibitor accumulates in the follicle during the growing stage (anagen), until it reaches a threshold level and so activity will stop and the follicle enters a resting stage (telogen). The inhibitor is then dissipated and activity recommences. Plucking the hair will remove the inhibitor with the club hair, therefore stimulating the follicle to start a new cycle. Swann (1958) believed that the cells are normally in a quiescent stage until an inductive stimulus is released which stimulates cellular activity. Bullough (1965) applied the chalone hypothesis to the cyclic growth of hair. The chalone is a protein produced by the differentiating part of the matrix and when its level is low the matrix cells go on dividing. But when a certain level has accumulated activity stops. Plucking a club hair will cause the removal of the differentiated matrix and so the chalone level will drop, resulting in commencement of the follicular activity.

Johnson & Ebling (1964) plucked rats' body hair during different phases of activity. Because of the differences in the time of eruption of the new hair, their results could not be explained by one of these theories; some of them could be explained by the inhibitor theory and some by the inductive stimulus theory. Johnson (1965), discussing the same results, believed that the factor influencing the follicles is more probably an inductive stimulus rather than an accumulation and dispersal of an

inhibitor. However, the results presented here show that the system is controlled by more than one factor. The inhibitor which is probably produced by the differentiated cells of the matrix drops into a critically low level after plucking and that will trigger the release of a stimulus, thus leading to the formation of a new hair. The difference between the results presented here and Johnson's results could be due to the fact that a different type of hair has been used. The resting period of vibrissae is very short. Body hairs have a relatively long resting period.

When a hair follicle was subjected to a non-epilating dose of irradiation, growth rate slowed for a few days, then started again and continued the same cycle (Wright, 1965). Probably less than 10% of the dividing matrix cells would be capable of indefinite cell division after a dose of 625 rads. In the case of plucking, only the upper differentiated half of the matrix was removed with the whisker, and all or most of the dividing part of the matrix cells was left behind. However, after plucking the follicle never continues the same cycle; it invariably rests for a fixed period then a new cycle starts. This strongly suggests the importance of the upper differentiating part of the matrix in the initiation and ending of a cycle. Removal of this part terminates the cycle.

If the inhibitor is formed by the differentiated cells of the matrix, plucking will remove the source of the inhibitor, the concentration may fall and thereby trigger the secretion of a stimulus which leads to the initiation of a new cycle. Another factor may be the loss of contact between the epithelium (differentiated matrix) and the mesenchyme (dermal papilla) which occurs after plucking but not irradiation.

ACKNOWLEDGMENTS

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REFERENCES

- BULLOUGH, W.S. (1965) Mitotic and functional homeostasis, a speculative review. *Cancer Research*, **25**, 1683.
- BUTCHER, E.O. (1936) Hair growth on skin transplants in the immature albino rat. *Anatomical Record*, **84**, 161.
- CHASE, H.B. (1955) The physiology and histochemistry of hair growth. *Journal of the Society of Cosmetic Chemists*, **6**, 9.
- COLLINS, H.H. (1918) Studies of normal moult and artificially induced regeneration of pelage in *Peromyscus*. *Journal of Experimental Zoology*, **27**, 73.
- DAVID, L.T. (1934) Studies on the expression of genetic hairlessness in the house mouse (*Mus musculus*). *Journal of Experimental Zoology*, **68**, 501.
- GHADIALLY, F.N. (1957) The effect of transposing skin flaps on the hair growth cycle in the rabbit. *Journal of Pathology and Bacteriology*, **74**, 321.
- IBRAHIM, L. & WRIGHT, E.A. (1975) The growth of rats and mice vibrissae under normal and some abnormal conditions. *Journal of Embryology and Experimental Morphology*, **33**, 831.
- JACKSON, D. & EBLING, F.J. (1972) The activity of hair follicles and their response to oestradiol in the guinea-pig *Cavia procollus* L. *Lancet*, **III**, 303.
- JOHNSON, B. & EBLING, F.J. (1964) The effect of plucking hairs during different phases of the follicular cycle. *Journal of Embryology and Experimental Morphology*, **12**, 465.
- JOHNSON, E. (1965) Inherent rhythms of activity in the hair follicle and their control. *Biology of the skin and hair growth*. Proceedings of a symposium held at Canberra, Australia, p. 491. Angus & Robertson.
- SILVER, A.F., CHASE, H.B. & ARSENAULT, C.T. (1967) Spontaneous and experimental hair growth of the mouse pinna. *Journal of Investigative Dermatology*, **48**, 444.
- SILVER, A.F. & CHASE, H.B. (1970) DNA synthesis in adult hair germ during dormancy (telogen) and activation (early anagen). *Developmental Biology*, **2**, 440.
- SWANN, M.M. (1958) The control of cell division—a review. II. Special mechanisms. *Cancer Research*, **18**, 1118.
- WRIGHT, E.A. (1965) The growth of mouse vibrissae. *Proceedings of the Royal Society of Medicine*, **58**, 804.

The long term effect of repeated pluckings on the function of the mouse vibrissal follicles

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SUMMARY

Single mouse vibrissae were plucked repeatedly at the same time of the cycle (10–15 days after eruption) for ten successive cycles from eight individual follicles. After three pluckings the first grey whisker appeared, after six pluckings all were grey.

Two follicles stopped producing whiskers after seven pluckings and another two after the 8th. Henceforth only 50% of the follicles continued producing whiskers until the end of the experiment. All the follicles which ceased to produce whiskers had a keratogenous cyst occupying most of the follicle in direct contact with the dermal papilla. In all cases the isolated dermal papilla was condensed and rounded in shape.

Both loss of pigment and cyst formation could be due to the mechanical damage in the follicle caused by repeated plucking.

Although the growth of all hairs is cyclic, passing through stages of activity and quiescence, a hair which is plucked at any stage of the cycle results in the eruption of a new hair after a predictable time. The effect of a single plucking on rat body hair during various stages of activity and quiescence has been studied by Johnson & Ebling (1964) and on mouse vibrissal follicles by Ibrahim & Wright (1978).

We have carried out successive pluckings of vibrissae during the anagen phase over a long period and made observations on the individual follicles.

MATERIALS AND METHODS

C₃H inbred mice born on the same day were used. Their E, F, G and H whisker follicles were therefore at the same stage of activity at the beginning of the experiment. Nomenclature and growth characteristics of the vibrissal follicles were described previously (Ibrahim & Wright, 1975). Sixteen vibrissal follicles, E, F, G and H on both sides of two female, 5-week-old, C₃H mice were used. The whiskers on the right side of both mice were plucked at the same time, and this process was repeated.

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every 3-4 weeks. After 10 successive pluckings the whiskers' final lengths and rate of growth for the next two generations were measured.

At the end of the experiment all the follicles were fixed and examined histologically. The plucked follicles were compared with their unplucked controls on the left side of the animal.

RESULTS

The whiskers subjected to the successive pluckings gradually lost their pigmentation. After three pluckings the first grey whisker was noticed. After six pluckings all were grey (Fig. 1). Two follicles stopped producing whiskers after the seventh plucking and another two after the eighth. Only half the follicles continued producing whiskers until the end of the experiment.

The final length of these whiskers varied between 19.8 and 24.8 mm (normal side 25.0-30.0 mm). Their rate of growth varied between 0.7 and 0.93 mm/day (normal side 0.78-1.0 mm/day). Fig. 2

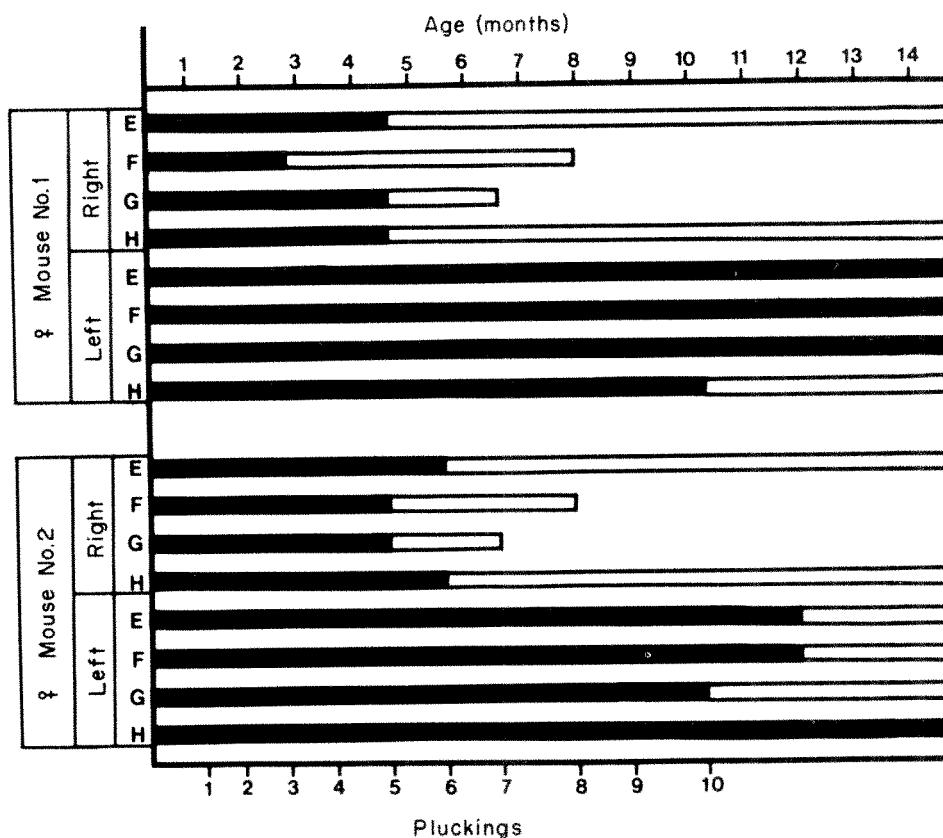


FIGURE 1. Growth and pigmentation of the sixteen whiskers (E, F, G and H on both sides of two female C₃H mice) during and after ten successive pluckings. The right side whiskers were plucked while the left side were growing normally. 50% of the normal whiskers were grey by 12 months of age while those subjected to the pluckings were all grey after 6 pluckings (at about 6 months of age).

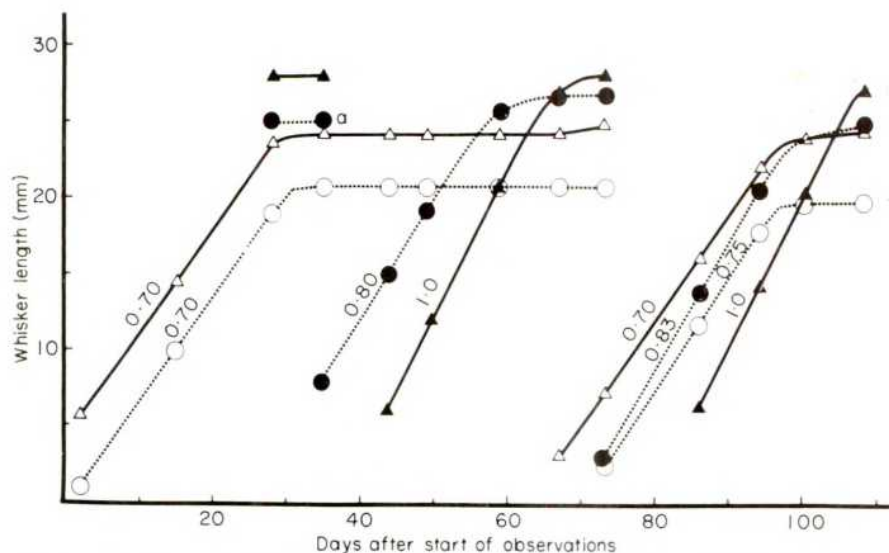


FIGURE 2. Growth curves of E and H vibrissae on the right side of mouse No. 1 which were still growing after ten pluckings. They were followed for two cycles. Each was compared with its normal twin on the left side of the mouse. The growth rates in mm/day were measured on the straight parts of the growth curves and are recorded on each slope. They were between 0.1 and 0.3 mm/day slower than their non-plucked counterparts.

a = tip of the vibrissa had been broken off when first observed.

(▲) left, control H; (△) right, experimental H; (●) left, control E; (○) right, experimental E.



FIGURE 3. (a) A longitudinal section through G follicle in one of the mice whose follicles were subjected to the ten successive pluckings and which stopped producing whiskers after the seventh plucking. The mouse was killed about 8 months later. The dermal papilla is in direct contact with the cyst structure extending along the follicle as high as the ring sinus (H & E, $\times 90$). An enlargement of the bottom part is shown in Fig. 3b where the dermal papilla is clearly of a rounded shape, very condensed cellularly and with some vacuoles in and outside this cellular condensation (H & E, $\times 500$).

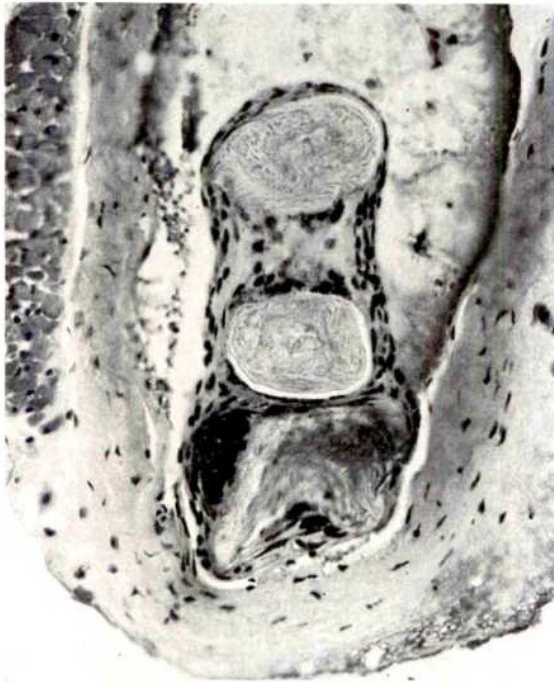


FIG. 4.

FIGURE 4. The bottom part of an F follicle which stopped producing whiskers after the eighth plucking. The mouse was killed 7 months later and the follicle was processed immediately. The cyst is also in direct contact with the dermal papilla (H & E, $\times 500$).



FIG. 5.

FIGURE 5. An E follicle, which continued producing whiskers after 10 successive pluckings, was sectioned after being followed up for two complete successive cycles and was at the early anagen stage of the third cycle (H & E, $\times 150$).

shows the final length and growth rate of two whiskers, still growing after 10 pluckings, plotted against their controls.

At the end of the two complete undisturbed cycles the mice were killed and all experimental follicles were studied histologically.

In all the follicles which ceased to produce hair, an isolated, rounded in shape and cellularly condensed dermal papilla was seen at the bottom of the follicle. Some cytoplasmic vacuoles within the condensed cells were seen in two follicles (Fig. 3a and 3b). There was no trace of the normal epidermal constituents of the follicles up to the level of the ringwülst. Small collections of keratogenous cells resembling small cysts were in direct contact with the outer root sheath and the dermal papillae, occupying the whole bottom half or two thirds of the follicle. One of these follicles is shown in Fig. 4. The follicles which continued producing whiskers until the end of the experiment showed dermal papillae of normal shape. One follicle is shown in Fig. 5.

DISCUSSION

Van Scott *et al.* (1968) noticed the tendency of hair follicles to produce keratin whenever disturbed

mechanically or chemically. Successive pluckings of actively growing hairs causes mechanical injury to the epidermal part of the follicle, and may also damage the tip of the dermal papilla. However, after a single plucking a variable number of matrix cells are left at the bottom end of the follicle and these cells should be capable of indefinite cell division. After multiple pluckings, in 50% of the cases the epithelium was diverted from hair formation into keratogenous cyst formation. The term keratogenous cyst is used descriptively to avoid suggesting too close a similarity to epidermal cyst, pilar cyst or pilomatrixoma as seen in humans. However, the finding of 'shadow cells' is suggestive of one of the regular features of the human pilomatrixoma (Lever & Schaumberg-Lever, 1975). Our findings are in accord with the concept that hair follicle epithelium is capable of different types of differentiation following mechanical disturbance.

The first change noticed was loss of pigment. This can be accounted for by mechanical loss of melanocytes (Potten *et al.*, 1971).

The failure of half the plucked follicles to produce hairs after 10 successive pluckings is difficult to explain in terms of damage to the dermal papilla as no significant loss of dermal cells was seen. Therefore the damage would seem to reside in the epithelial cells of the follicle. One hypothesis to fit the facts would be that the potential for the specialized differentiation and cell division of the epithelial cells is limited. Ten successive pluckings might exhaust this potential (Horton & Whitely, 1969).

It has been shown that the heavily irradiated and permanently epilated vibrissal follicles do not lose their dermal papillae and these irradiated dermal papillae were able to induce new additional hairs when transplanted into normal follicles (Ibrahim & Wright, 1977), which proved that permanent epilation was because of the massive damage inflicted on the follicular epithelium.

Histological features of the dermal papillae in the follicles which ceased to produce whiskers after ten successive pluckings were very similar to those of highly irradiated dermal papillae, rounded in shape, cellularly condensed and with very little intercellular substance. However, the surrounding matrix epithelium being diverted into keratogenous cyst formation appears to be the reason for the permanent epilation in this case. By analogy it may be argued that plucking somehow detaches the effective epithelium from the dermal papilla but from histological examination of plucked follicles it would appear that this is not simply stripping of the epithelium of the follicle as both outer root sheath and the lower matrix cells remain.

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REFERENCES

- HORTON, D.L. & WHITELEY, H.J. (1969) The effect of age on hair growth in CBA mouse. Observations on repeating plucking. *Journal of Gerontology*, **24**, 324.
- IBRAHIM, L. & WRIGHT, E.A. (1975) The growth of rats and mice vibrissae under normal and some abnormal conditions. *Journal of Embryology and Experimental Morphology*, **33**, 831.
- IBRAHIM, L. & WRIGHT, E.A. (1977) Inductive capacity of irradiated dermal papillae. *Nature*, **265**, 733.
- IBRAHIM, L. & WRIGHT, E.A. (1978). The effect of a single plucking at different times in the hair cycle on the growth of individual mouse vibrissae. *British Journal of Dermatology*, **99**, 363.
- JOHNSON, E. & EBLING, F.J. (1964) The effect of plucking hairs during different phases of the follicular cycle. *Journal of Embryology and Experimental Morphology*, **12**, 465.
- LEVER, W.F. & SCHAUMBERG-LEVER (1975) *Histopathology of the Skin*, 5th edn. Lippincott, Philadelphia.

- POTTEN, C.S., MERKOW, L.P. & PARDO, M. (1971) Ultrastructural changes in mouse hair follicle pigment cells after plucking, X-rays and Actinomycin D treatment. *Laboratory Investigation*, **25**, 607.
- VAN SCOTT, E.J. & FLAXMAN, B.A. (1968) Environmental control of epithelial cells *in vivo* and *in vitro*. In: *Epithelial-mesenchymal Interactions* (Ed. by R.Fleischmayer and R.E.Billingham), p. 280. Williams & Wilkins, Baltimore.

The aniline blue fluorescence staining of eosinophilic granulocytes

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SUMMARY

An aniline blue fluorescence staining technique specific for eosinophils was compared to haematoxylin and eosin to determine the percentages of eosinophils in the peripheral blood of patients with atopic or with allergic contact dermatitis. The aniline blue technique proved to be excellent for demonstrating eosinophilic granulocytes. Although the eosinophilic granulocytes were reasonably demonstrable in thin layers stained with haematoxylin and eosin, the aniline blue staining seems to be better in cases of doubt.

Using the same techniques the presence of eosinophil granulocytes was studied in 5 punch biopsies taken from positive patch tests (after 48 h) and 5 biopsies from positive skin reactions after an intradermal injection with an atopic allergen (after 20–30 min). In all punch biopsies taken after an intradermal injection with an atopic allergen, eosinophilic granulocytes, often numerous, were seen in the vessels of the dermis. In punch biopsies taken from positive patch tests the eosinophilic granulocytes were seen scattered through the dermis in variable numbers.

The eosinophilic granulocyte is morphologically a polymorphonuclear leukocyte like the basophilic and neutrophilic granulocytes. The cell has a round, sometimes slightly oval form and its diameter is 12–17 μm . The mature cell usually has a bilobed, sometimes a trilobed nucleus. Paul Ehrlich first distinguished the coarsely grained eosinophilic granulocyte from other white blood cells by an acid dye, eosin (Ehrlich, 1879). The eosinophilic granulocytes were thought to play a role in allergic reactions as they were often found in the tissues involved in these inflammatory processes.

The purpose of this study is to examine the presence of eosinophilic granulocytes in immediate type allergic reactions and in cell-mediated allergic reactions. As conventional haematoxylin and eosin stains may not always show eosinophils clearly, we tested the aniline blue fluorescence stain of Eder (1960). Aniline blue is an acid dye like eosin.

Eosinophils of peripheral blood of 5 healthy department members, 5 patients with atopic dermatitis and 5 patients with allergic contact dermatitis were obtained by the method of Day (1970) and the percentages of the eosinophils were compared, using the haematoxylin and eosin and the aniline blue fluorescence staining techniques.

MATERIALS AND METHODS

Eosinophils were obtained from five patients with atopic dermatitis, five patients with allergic contact dermatitis and from five normal healthy department members as controls.

Punch biopsies (five) were taken from positive skin reactions to an atopic allergen (after 20–30 min) and from positive patch tests (five) after 48 h. The various antigens to which the two groups of patients were sensitive are listed in Tables 2 and 3. Punch biopsies taken from homologous uninvolved skin were used as control.

TABLE 1. Results of aniline blue fluorescent and H & E staining in isolated eosinophilic granulocytes

		Aniline blue staining		H & E staining		
		Eosinophilic granulocytes (%)	Other cells (%)	Eosinophilic granulocytes (%)	Other cells (%)	Possible eos. granulocytes (%)
Healthy controls (5)	Mean	49.2	50.8	43.6	46.0	10.4
	Range	39–62	46–61	29–57	35–55	9–16
Allergic contact dermatitis (5)	Mean	58.6	41.4	50.6	39.2	10.2
	Range	36–39	31–64	23–62	30–60	5–17
Atopic dermatitis (5)	Mean	54.2	45.8	49.8	40.6	8.4
	Range	35–70	30–65	29–68	23–62	4–11

TABLE 2. Results of aniline blue fluorescent and H & E staining in patch test biopsy sections of five patients (taken after 48 h)

Case	Antigen	Aniline blue fluorescent staining	H & E staining
1	Tincture of iodide 0.5%	++	+
2	Potassium dichromate 0.5% in vaseline	++	+
3	Nickel sulphate 2.5% in vaseline	+	—
4	Balsam of Peru 25% in vaseline	++	++
5	Formaldehyde 0.5% in vaseline	++	+

—, Negative, less than 5 eosinophilic granulocytes per section.

+, 5–20 eosinophilic granulocytes per section.

++, More than 20 eosinophilic granulocytes per section.

Chemicals and reagents

(1) Isopaque (Sodium N-ethyl-3,5-diacetamido-2,4,6-triiodbenzoate) solution. 20 ml Isopaque (Nyegaard and Co, Oslo, Norway) was diluted with 25 ml distilled water.

(2) Isopaque-dextran mixture. Ten parts Isopaque solution were mixed with 24 parts 6% dextran (Pharmacia, Upsala, Sweden) in isotonic NaCl.

(3) Isopaque-distilled water mixture ($d=1.148$). Ten parts of Isopaque were mixed with 20.9 parts distilled water.

(4) Phosphate buffer solution (PBS), pH 7.2: 8.5 g NaCl + 1.07 g Na_2HPO_4 + 0.39 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ are dissolved in 1 litre distilled water.

(5) Aniline blue solution. Aniline blue, soluble in water (Merck, Darmstadt, Germany) was dissolved in 1000 parts of distilled water.

TABLE 3. Results of aniline blue fluorescent and H & E staining in 5 biopsy sections from a positive skin reaction after an intracutaneous injection with an atopic inhalant allergen (taken after 20-30 min)

Case	Allergen*	Aniline blue fluorescent staining	H & E staining
1	Pollen 100 NE/ml	++	++
2	Human dander 0.1%	+	+
3	Human dander 0.1%	++	++
4	Mixed animal epidermal products 0.01%	++	++
5	Human dander 0.1%	++	++

—, Negative, less than 5 eosinophilic granulocytes per section.

+, 5-20 eosinophilic granulocytes per section.

++, More than 20 eosinophilic granulocytes per section.

* NV Haarlems Allergenen Laboratorium, Harlem, The Netherlands.

Microscopy

Light microscopy. A Leitz Orthoplan microscope with a 16 V and 15 W lamp and a Leitz substage condensor were used.

Fluorescent microscopy. A Leitz Orthoplan microscope with a Xenon XBO lamp and a Leitz vertical illuminator was used. Aniline blue fluorescence was detected using UV broad-band epillumination obtained with the filter combination 2 × UG1 (2 mm).

Leitz water immersion objectives (× 50 and × 90) were used in all cases.

The separation of eosinophilic granulocytes from peripheral blood

The eosinophilic granulocytes were separated by the method of Day (1970) with some modifications. The separation of different white blood cells is based on differences in density. The removal of red cells depends on properties of the cell clumping mixture, such as its density, viscosity and osmolarity. It is further influenced by blood volume, dilution of blood and temperature. The clumping process is made more efficient when the procedure takes place at an interface. Therefore we layered the collected blood on an Isopaque-Dextran 6% mixture instead of making a solution of blood and dextran as used by Day (1970).

Thirty ml of venous blood was collected and defibrinated in an Erlenmeyer flask containing twenty-five glass pearls. After defibrinating the blood was divided into five parts of 6 ml and layered on top of 6 ml Isopaque-Dextran 6% mixture in tubes with a diameter of 25 mm and left for 50 min. The red cells are clumped by Isopaque-Dextran mixture at the interface and sedimented at the bottom of the tube at 1 × g gravity field. The leukocyte-rich plasma layer was pipetted off and carefully layered on an Isopaque-distilled water mixture (d = 1.148 g/ml) in tubes with a diameter of 13 mm. The volume of the leukocyte-rich plasma layered on top was 4 ml at most. Each tube contained 4 ml Isopaque-distilled water mixture. This suspension was centrifuged at 400 $\bar{g}$ for 40 min. After centrifugation the clear plasma layer and the interface, where most of the leukocytes remain, were pipetted off, leaving an eosinophil-rich pellet. These eosinophil-rich pellets were washed three times at 200 $\bar{g}$ for 5 min with isotonic NaCl. For all separations only thoroughly cleaned and siliconized glassware was used.

Aniline blue fluorescence staining and examination of the isolated eosinophilic granulocytes

The aniline blue fluorescence staining method of Eder (1960) is probably based on a histochemical reaction between an unknown substance of the eosinophilic granules and the acid dye aniline blue, as

aniline blue alone gives no fluorescence. This histochemical reaction is characteristic for the granules of eosinophilic granulocytes. Granules of neutrophilic or basophilic granulocytes give no reaction (Eder, 1960). The staining of the isolated eosinophilic granulocytes was carried out as follows. To determine the percentage of eosinophilic granulocytes the object slides were initially cleaned in 96% ethanol and then passed through a gas flame. One drop of the eosinophilic granulocyte suspension was sufficient for a thin cell layer. These layers were air dried and subsequently fixed by means of 70% ethanol. Immediately afterwards the slides were stained in the aniline blue solution for three minutes. To distinguish the eosinophilic granulocytes from other cells, the nuclei of all the cells present were stained with acridine orange 1/10,000 for 4 s after the aniline blue staining. Fluorescence examination was carried out on the cell layers. The eosinophilic granulocytes showed a coarsely grained blue staining. Non-eosinophilic cells did not show any stained cytoplasm. The nuclei of the cells fluoresced yellow-green. With this method there were no doubtful cells. For comparison, haematoxylin and eosin staining was carried out. On each slide 200 cells were counted. Cells with a bilobed nucleus but no conspicuous granules, and cells with distinct granules but no bi- or trilobed nucleus, were called possible eosinophilic granulocytes.

The preparation of skin biopsies

Punch biopsies were taken from positive patch tests or positive skin reactions to an atopic inhalant allergen (Tables 2 and 3) under local anaesthesia (2% xylocaine with 1/80,000 adrenaline). The specimens were prepared according to the techniques described earlier (Cormane, Szabo & Hauge 1970) which involves placing the tissue sample into an empty capsule and then filling the capsule with a 10% gelatin solution (Difco Labs, Detroit, U.S.A.). The capsule is then placed in a small tin container, frozen in liquid nitrogen and stored at -70°C .

Sections were cut at $4\text{ }\mu\text{m}$ with a cryostat (International Equipment Co, Washington DC, U.S.A.). From each biopsy two successive sections were studied with the aniline blue fluorescence technique and the haematoxylin and eosin technique.

The aniline blue staining was carried out by staining sections for 3 min in the aniline blue solution. The appearance of eosinophilic granulocytes in the vessels and in the dermis was studied. The specimens were considered negative (—) if there were less than five eosinophilic granulocytes per section. A count of 5–20 eosinophilic granulocytes in a section was considered as a moderate number (+) and more than 20 eosinophilic granulocytes was considered as numerous (++). Normal uninvolved skin was judged according to the method.

RESULTS

Isolated eosinophilic granulocytes

The percentages of eosinophilic granulocytes isolated from the peripheral blood of healthy controls, atopic patients and patients with allergic contact dermatitis are summarized in Table 1. The mean percentages of eosinophilic granulocytes determined with the aniline blue fluorescence technique were 49.2% in healthy controls, 58.6% in patients with allergic contact dermatitis and 54.2% in patients with atopic dermatitis. There were no doubtful cells. The mean percentages determined with haematoxylin and eosin staining were 43.6%, 50.6% and 49.8% respectively. The mean percentages of possible eosinophilic granulocytes were 10.4% in controls, 10.2% in patients with allergic contact dermatitis and 8.4% in patients with atopic dermatitis.

The sum of the mean percentages of possible eosinophilic granulocytes and eosinophilic granulocytes in controls (54.0%) as well as in patients with allergic dermatitis (60.8%) and in patients with atopic dermatitis (58.2%) is higher than the percentages of eosinophilic granulocytes found with the aniline blue fluorescence technique.

The presence of eosinophilic granulocytes in the skin specimens

All punch biopsies of the uninvolved homologous skin failed to show any eosinophilic granulocytes. The results of the determination of eosinophilic granulocytes in the punch biopsies taken from positive patch tests are summarized in Table 2. The detection and counting of the eosinophilic granulocytes with the aid of the specific aniline blue fluorescence technique was much easier than with the H & E staining technique. The eosinophilic granulocytes were found to be scattered through the tissues, sometimes in the epidermis up to the stratum corneum. The bright fluorescence of the granules of the eosinophilic granulocytes is obvious. Eosinophilic granulocytes were much more difficult to detect with the H & E staining.

As in the sections taken from positive skin reactions to atopic allergens the infiltrate of eosinophilic granulocytes was limited mainly to the dermal vessels (Fig. 2) and there often was a predominance of

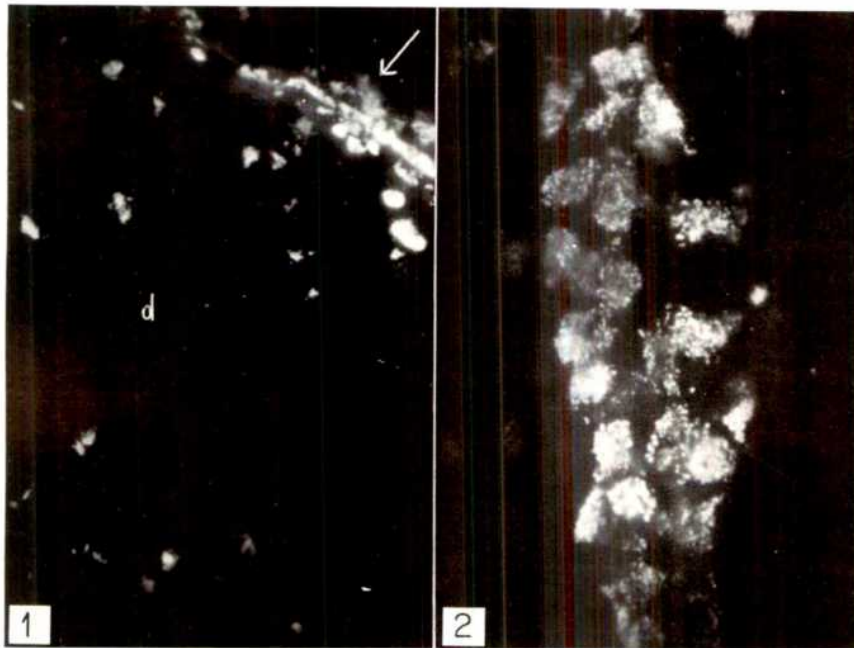


FIGURE 1. Cryostat section of a biopsy taken from a positive patch test (after 48 h) (Aniline blue staining, $\times 400$). Note the eosinophilic granulocytes scattered through the dermis (d) up to the stratum corneum. $\uparrow$.

FIGURE 2. Cryostat section of a biopsy taken from a positive skin reaction after an intracutaneous injection with atopic inhalant allergen (after 20–30 min) (Aniline blue staining, $\times 720$). Note the fluorescent granules of the eosinophilic granulocytes in an intradermal vessel.

eosinophilic granulocytes, the differences in counting were less evident (Table 3). However, the aniline blue staining technique gives here also an excellent demonstration of the immediate recruiting of eosinophilic granulocytes in atopic reactions.

DISCUSSION

As histological studies on the appearance of eosinophilic granulocytes in inflammatory reactions with H & E staining techniques are sometimes imperfect (Medenica & Rostenberg, 1971) and also a specific eosinophilic granulocyte staining can sometimes meet technical difficulties (Steigleder & Inderwisch, 1975; Eder, 1960), we tested the specific aniline blue fluorescence staining of Eder (1960) on isolated eosinophilic granulocytes as well as in biopsies taken from immediate type- and cell mediated allergic skin reactions.

Before examining the tissue sections we tested the aniline blue fluorescence staining on isolated eosinophilic granulocytes from the peripheral blood. So far as we know this staining technique with the method of Eder (1960) has not often been used in histological studies. The reason might be that a fluorescence microscope is needed. As nowadays immunofluorescence studies are widely used in many laboratories the application of this fluorescence staining technique will present no difficulties.

The method proved to be excellent in comparison with H & E staining techniques as there were no cases of doubt. The percentages of eosinophilic granulocytes we obtained (49.2, 58.6 and 54.2%) are lower than those of Day in 1970 (90%). Maybe this was due to our modification but we preferred to keep the contamination by erythrocytes as low as possible. When the mean percentages of possible eosinophilic granulocytes and eosinophilic granulocytes are added, the figures of 54.0, 60.8 and 58.2% are apparently better. The fact that Day (1970) did not describe his staining technique for the isolated eosinophilic granulocytes hampers a reliable comparison of his and our results.

There is some confusion about the presence of eosinophilic granulocytes in positive patch tests. According to some authors these are rarely seen (Lever 1975). Medenica and Rostenberg stated that basophilic granulocytes (which can resemble eosinophilic granulocytes) form an important part of the cellular infiltrate. Also the time at which the biopsies were taken can be responsible for differences. We took our biopsies after 48 h. With the aniline blue fluorescence staining we found many eosinophils in 4 out of 5 patients (Table 2).

In the five biopsies taken from the immediate type allergic skin reactions there was much more concordance between the aniline blue fluorescence technique and the H & E technique (Table 3). The interpretation was not hampered by an abundant heterogenous infiltrate. The cellular reaction was limited mainly to the blood vessels and consisted predominantly of eosinophilic granulocytes whose presence could easily be demonstrated by means of the H & E staining technique.

In conclusion we advocate the use of the aniline blue staining technique to demonstrate eosinophilic granulocytes in tissue sections and in monolayer preparations.

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REFERENCES

- CORMANE, R.H., SZABO, E. & HAUGE, L.S. (1970) Immunofluorescence of the skin. The interpretation of the staining of blood vessels and connective tissue aided by new techniques. *British Journal of Dermatology*, **82**, suppl. 5, 26.
- DAY, R.P. (1970) Eosinophil cell separation from human peripheral blood. *Immunology*, **18**, 955.
- EDER, H. (1960) Fluoreszenzmikroskopische Eosinophilen-Diagnostik. *Leitz Mitteilungen*, Band 1, no. 4, 113.
- EHRLICH, P. (1879) Über die speziellen Granulationen des Blutes. *Archiv für Anatomie und Physiologie. Physiologische Abteilung*, 571.
- LEVER, W.F. & SCHAUMBURG-LEVER, G. (1975) *Histopathology of the Skin*, 5th edn, p. 100. Lippincott Co. Philadelphia, Toronto.
- MEDENICA, M. & ROSTENBERG, A. (1971) A comparative light and electron microscopic study of primary irritant contact dermatitis and allergic contact dermatitis. *Journal of Investigative Dermatology*, **56**, 259.
- PARISH, W.E. (1970) Investigations on eosinophilia. The influence of histamine, antigen-antibody complexes containing γ_1 or γ_2 globulins, foreign bodies (phagocytosis) and disrupted mast cells. *British Journal of Dermatology*, **82**, 42.
- STEIGLEDER, G.K. & Inderwisch, R. (1975) Eosinophile Granulocytes in Effloreszenz bei Psoriasis und Neurodermatitis constitutionalis (atopische Dermatitis). *Archives of Dermatology*, **254**, 253.

Alopecia areata: immunofluorescence and other studies

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SUMMARY

In a study of 24 patients with alopecia areata, scalp biopsies were examined for immunofluorescence; all were negative. A number of other tests also failed to confirm an auto-immune aetiology for this disease. A possible explanation lies in the heterogeneity of alopecia areata, only a small proportion of cases being of the auto-immune type.

It is commonly held that alopecia areata (AA) is an autoimmune disease; nevertheless, the evidence is inconclusive (Rook, 1977). Associated findings have included the presence of thyroid disease (Cunliffe *et al.*, 1969; Muller & Winkelmann, 1963) and of vitiligo (Anderson, 1950). An increase in auto-antibodies in AA was found by Main *et al.* (1975), Kern *et al.* (1973) and Du Vivier & Munro (1975). On the other hand, Cunliffe *et al.* (1969), Betterle *et al.* (1975) and Muller & Rook (1976) found only the normal incidence of auto-antibodies while Salamon, Musafija & Milicerie (1971) and Main *et al.* (1975) found no increase in incidence of thyroid disease in patients with AA.

PATIENTS AND METHODS

We studied 24 patients with AA. They were consecutive unselected patients, 12 male and 12 female. The men were aged between 14 and 57 (mean 38 years) and the women were from 16 to 60 (mean 32 years). Eleven patients had a history of 1 year or less, while 13 had alopecia of longer duration up to 22 years; the mean duration was 4.7 years. The extent of the involvement varied from a single area to several areas on the scalp (the majority of cases); two cases had alopecia totalis and two alopecia universalis.

A full history was taken with special reference to past or family history of organ-specific auto-immune disease and the patients were examined clinically. Blood was then taken for the following investigations: haemoglobin, white cell count, erythrocyte sedimentation rate (E.S.R.) and blood film, serum B12 and folic acid, liver function tests (bilirubin, alkaline phosphatase, calcium and phosphate, total protein, albumin and aspartate amino-transferase), thyroid function tests (serum thyroxine, T3

uptake, free thyroxine index (FTI), thyroid stimulating hormone level (TSH) and, in 6 cases, the serum tri-iodothyronine level), blood sugar, protein electrophoresis and immunoglobulin levels. In addition, standard indirect immunofluorescent techniques were used to detect anti-nuclear factor (ANF) and rheumatoid factor and antibodies to mitochondria, thyroid epithelium and thyroid colloid, gastric parietal cells and intrinsic factor, skeletal and smooth muscle and renal glomerular basement membrane.

A scalp biopsy was performed, with the patients' informed consent, and half the specimen was examined histologically while half was snap-frozen in liquid nitrogen for immunofluorescence studies. These were performed using standard direct methods with fluorescein-labelled sheep anti-human IgG, IgM and IgA (Wellcome), goat anti-human C₃ (Hyland) and rabbit anti-human fibrinogen (Wellcome).

RESULTS

The personal histories were unrevealing: two patients had previous vitiligo, five attributed the onset of their alopecia to severe emotional shock and one to physical trauma. Twelve patients had a family history of alopecia areata, four of diabetes mellitus, two of vitiligo, two of thyroid disease and one each of pernicious anaemia and rheumatoid arthritis.

The blood count, film appearances and ESR were all normal. One patient had a low serum B₁₂ on three occasions (120, 150 and 120 pg/ml). Folic acid levels and liver function tests were within normal limits. Thyroid function tests, blood sugar and protein electrophoresis showed no significant abnormality. Immunoglobulin levels were as follows:

IgG 1092 mg% $\pm$ 266 (NR 500-1700).

IgA 232 mg% $\pm$ 110 (NR 140-420).

IgM 201 mg% $\pm$ 137 (NR 50-200).

Positive auto-antibodies were as follows:

Smooth muscle one case at a titre of 1 in 10.

Thyroid colloid one case at a titre of 1 in 320.

Gastric parietal one case positive together with a weakly positive thyroglobulin titre of 1 in 10.

ANF one case at a titre of 1 in 80.

Mitochondria one case weakly positive (negative when repeated).

Skeletal muscle one case weakly positive.

No immunofluorescence of the scalp biopsies was detected to IgG, IgA, IgM, C₃ or fibrinogen.

The commonest histological finding was a variable degree of atrophy of the hair follicles (14 out of 24 cases) with a surrounding infiltrate which consisted of lymphocytes alone in seven cases and of mixed cell types in ten cases. There were only two instances of plasma cell infiltrate. Six biopsies showed a fibrous tissue reaction around the deeper part of the follicular sheaths. In three cases the affected follicles lay higher in the dermis; the same number showed follicular plugging. One case of alopecia universalis had no apparent abnormality of the hair follicles.

DISCUSSION

Several studies have shown an increased incidence of thyroid disease in patients with AA, ranging from 33% in females (Cunliffe *et al.*, 1969) to 8% of the 736 patients reported by Muller & Winkelmann (1963). However, neither Salamon, Musafija & Miliceric (1971) nor Main *et al.* (1975) could confirm these findings. None of our patients was known to have thyroid disease and only two (8%) gave a family history of this.

Two patients had vitiligo (8%); whereas the larger series of Muller & Winkelmann (1963) and of Anderson (1950) had vitiligo associated in 4%.

We found a family history of AA in 12 patients (50%); this is an exceptionally high figure. Cunliffe *et al.* (1969) obtained such a history in 25%; Muller & Winkelmann (1963) in only 10% of their series.

Hooper *et al.* (1972) studied auto-immunity in a homogeneous Caucasian rural community in Australia: they found that 21.6% of the population had at least one auto-antibody detectable, while 3.6% had more than one. The figures for females alone were 27.5% and 5.5% respectively. Dingle *et al.* (1966) found thyroglobulin antibodies at a titre of 1 in 25 or more in 16.2% of women and 4.3% of men in a random sample of 469 patients from general practice in North East England.

Our figure of 25% (6 out of 24) with auto-antibodies and 4% (one patient) with two auto-antibodies detected is in accord with the normal population studies. Three other series had found no increase in auto-antibodies in AA (Cunliffe *et al.*, 1969; Betterle *et al.*, 1975; Muller & Rook, 1976). On the other hand, Main *et al.* (1975) found a significant increase in smooth muscle antibodies and Kern *et al.* (1975) reported significant increases in thyroid antibodies (present in 25%), parietal cell antibodies (10%) and adrenal cell antibodies (7.5%). Du Vivier & Munro (1975) in a large study of alopecia areata in mongols found eight out of 23 female mongols had thyroid antibodies (35%) compared to two of 23 age-matched controls.

Full standard immunofluorescent studies have not previously been reported. Kern *et al.* (1973), using only anti IgG sera, obtained entirely negative results. Cochrane *et al.* (1976) reported a comprehensive auto-antibody profile showing no statistically significant increase in circulating auto-antibodies. However, they did no direct immunofluorescence studies as they considered it unethical to take scalp biopsies.

The 24 scalp biopsies that we took were examined using standard techniques in the immunology departments of both hospitals and no immunofluorescence was found.

AA will continue to be regarded as a probable auto-immune disorder because of its association with organ-specific auto-immune disease, because of the characteristic histopathology, and because of its response to corticosteroid therapy. Why, then, were our results apparently so strongly against such a theory? The most likely explanation lies in the work of Ikeda and of Rook.

Ikeda (1965) presented the results of a survey of 1989 patients from Japan over an 18 year period. She classified them into four groups: Group I, the common type, formed 83% of the whole series; Group II, the atopics, were 10%; Group III, the so-called prehypertensive, were 4%; while Group IV, the combined (auto-immune) group were only 3% of the whole.

Rook (1976) reported on a personal series of 200 patients followed up for at least 10 years. He was doubtful about Ikeda's prehypertensive group but was able to classify his patients broadly into three groups—those with a personal (or family) history of atopy; those with a personal or family history of auto-immune disease; the third group had no such history.

Penders (1968) used a somewhat similar classification but, somewhat surprisingly, found that the majority (52.4%) of his patients were atopic.

It is, therefore, entirely possible that our small series might not have included any of the 3% of those with alopecia areata of auto-immune type and that it represented mainly the common type of Ikeda.

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REFERENCES

- ANDERSON, I. (1950) Alopecia areata: a clinical study. *British Medical Journal*, **ii**, 1250.
- BETTERLE, C., PESSERICO, A., DAL PRETE, G. & TRISOTTO, A. (1975) Antibodies in alopecia areata. *Archives of Dermatology*, **111**, 927.
- COCHRANE, R.E.I., THOMSON, J. & MACSWEEN, R.N.M. (1976) An auto-antibody profile in alopecia totalis and diffuse alopecia. *British Journal of Dermatology*, **95**, 61.
- CUNLIFFE, W.C., HALL, R., STEVENSON, C.J. & WEIGHTMAN, D. (1969) Alopecia areata, thyroid disease and auto-immunity. *British Journal of Dermatology*, **81**, 879.
- DINGLE, P.R., FERGUSON, A., HORN, D.B., TUBMEN, J. & HALL, R. (1966) The incidence of thyroglobulin antibodies and thyroid enlargement in a general practice in North East England. *Clinical and Experimental Immunology*, **1**, 277.
- DU VIVIER, A. & MUNRO, D.D. (1975) Alopecia areata, auto-immunity and Down's syndrome. *British Medical Journal*, **i**, 191.
- HOOPER, B., WHITTINGHAM, S., MATTHEWS, J.D., MACKAY, I.R. & CURNOW, D.H. (1972) Autoimmunity in a rural community. *Clinical and Experimental Immunology*, **12**, 79.
- IKEDA, T. (1965) A new classification of alopecia areata. *Dermatologica*, **131**, 42.
- KERN, F., HOFFMANN, W.H., HAMBRICK, G.W. & BUZZARD, R.M. (1973) Alopecia areata: immunologic studies and treatment with prednisone. *Archives of Dermatology*, **107**, 407.
- MAIN, S.A., ROBBIE, R.B., GRAY, E.S., DONALD, D. & HORNE, C.H.W. (1975) Smooth muscle antibodies and alopecia areata. *British Journal of Dermatology*, **92**, 389.
- MULLER, K. & ROOK, A.J. (1976) Cited by Rook (1977).
- MULLER, S.A. & WINKELMANN, R.K. (1963) Alopecia areata. *Archives of Dermatology*, **88**, 290.
- PENDERS, A.J.M. (1968) Alopecia areata and atopy. *Dermatologica*, **136**, 395.
- ROOK, A.J. (1976) Cited by Rook (1977).
- ROOK, A.J. (1977) Common baldness and alopecia areata. In: *Recent Advances in Dermatology* (Ed. by A.J.Rook), chpt. 7, p. 241. Blackwell Scientific Publications, Oxford.
- SALAMON, T., MUSAFIJA, A. & MILICERIC, M. (1971) Alopecia Areata und Erkrankungen der Thyreoidea. *Dermatologica*, **142**, 62.

A quantitative study of membrane coating granules in follicles undergoing experimental comedo formation

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SUMMARY

Membrane coating granules (MCGs) may be required for normal keratinization. Comedone formation is associated with abnormal keratinization, and so the MCGs in sebaceous follicles were counted during experimental comedogenesis in the rabbit. During induced comedo formation with 50% oleic acid in paraffin oil, the numbers of MCGs decreased significantly; first, at the epidermis and mouth of the follicle and then later, at the follicular canal and common sebaceous duct. The maximum decrease of about 40% was reached by the 6th day in these regions. Throughout the treatment, the MCG count in the individual sebaceous ducts remained fairly constant.

In 1974, Knutson reported an apparent decrease in the number of MCGs in follicles containing a comedo. He observed that in normal follicles there were large and numerous clusters of MCGs present in the follicular canal and sebaceous ducts. However, during comedo formation, when the walls of the follicular canal and sebaceous ducts underwent cohesive hyperkeratinization (Kligman, 1974), they became less apparent. He suggested that this reduction in the numbers of MCGs may have caused the horny cells to stick together and compact in the canal to form a comedo—the primary lesion of acne.

Recently, an apparent decrease in the numbers of MCGs was found in a study of cohesion of horny cells during experimental comedo formation (Woo-Sam, 1977). The present study was undertaken to discover whether this decrease was a true one.

MATERIALS AND METHODS

Comedones were induced with 50% oleic acid in hair follicles in the external ear canal of 30 rabbits and the tissue was processed for electron microscopy as previously described (Woo-Sam, 1977). The rabbits were divided into groups of 5, and biopsies were taken from each group after 2, 4, 6, 9, 14 and

21 days' treatment respectively. Ten follicles from each group of treated animals and 5 control follicles which were treated with paraffin oil alone were sampled. Follicles were chosen at random, orientated and cut to produce a 1 μ m thick longitudinal section of the entire follicle. Ultrathin sections of the whole follicle were then cut for electron microscopy.

Electron micrographs were taken from the regions as shown in Fig. 1. The electron microscope was calibrated with a cross grating so that the exact magnification was known. The original magnification was $\times 5000$ and plates were enlarged to give a final print magnification of $\times 20,000$. A grid of 4 cm squares was superimposed photographically on to the print so that the MCGs could be counted more accurately. It was arranged with 5 squares horizontally and 6 squares vertically and the horizontal lines as parallel as possible to the stratum corneum (Fig. 2).

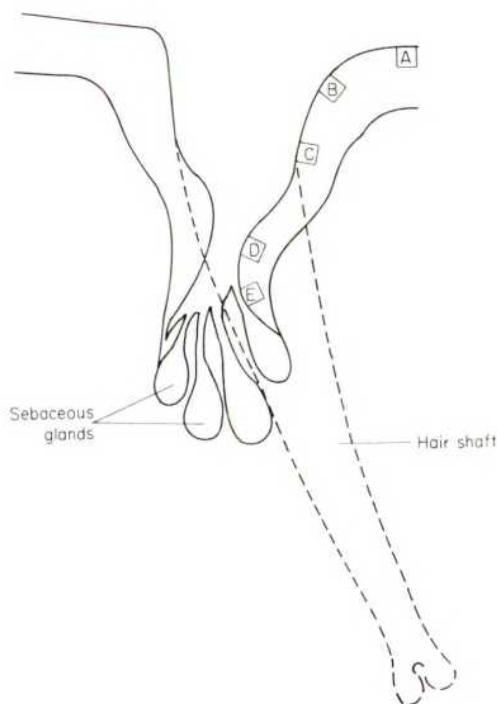


FIGURE 1. Diagram of typical hair follicle to show areas studied by electron microscopy. A, wall of epidermis; B, wall of mouth of follicle; C, wall of follicular canal; D, wall of common sebaceous duct; E, wall of individual sebaceous duct.

Only MCGs present in the cytoplasm of keratinocytes were counted: those already secreted into the intercellular spaces were ignored.

Random photographs within the areas indicated in Fig. 1 were recorded, since it was noticed that the distribution of the MCGs was not homogenous. Altogether 450 photographs were examined.

RESULTS

More MCGs were present in the lower layers of the stratum granulosum than in the upper layers, which were about to undergo keratinization (Fig. 3). Two different types of MCGs were seen (Fig. 4): one with the typical lamellar structure (Fig. 4a) (Odland, 1960) and the other, which was oval or

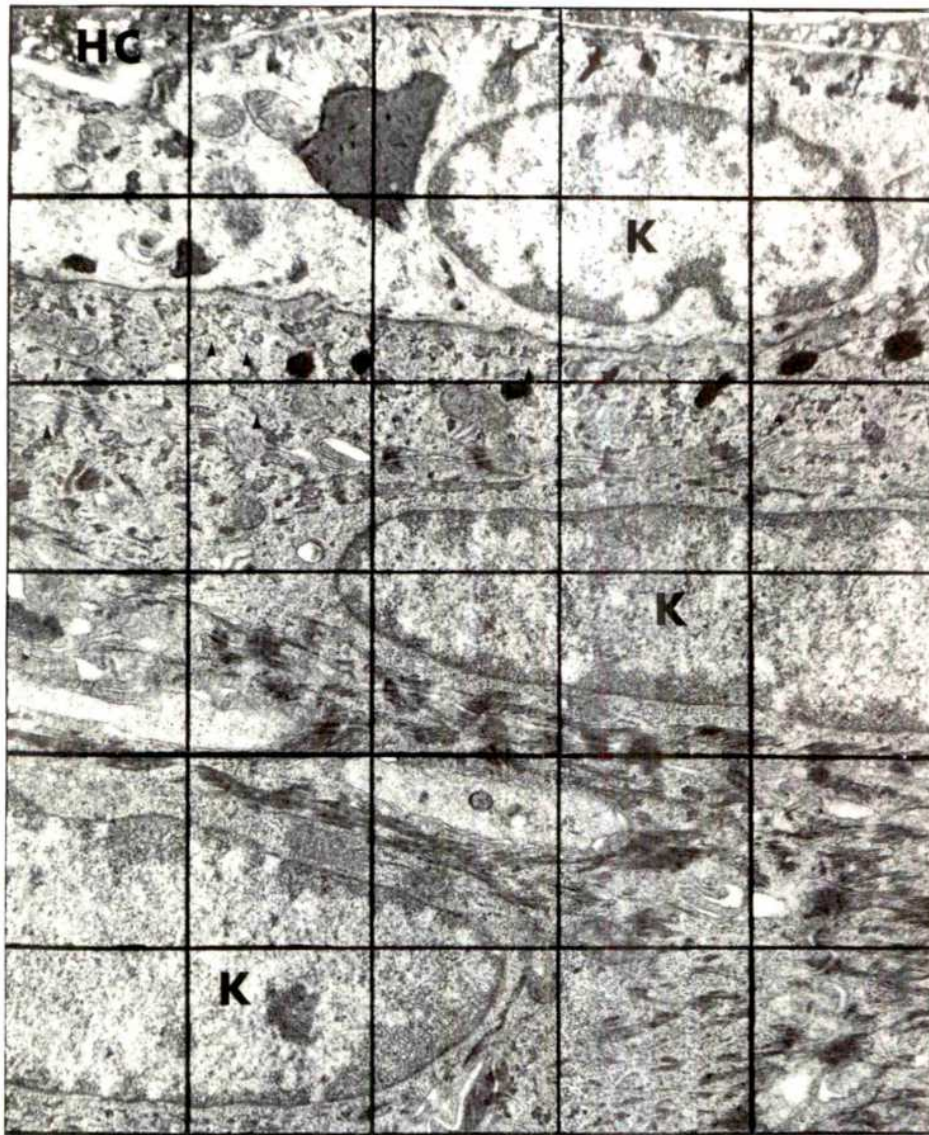


FIGURE 2. Superimposed grid ($\times 15,000$). HC, horny cell; K, keratinocyte; Arrows indicate some MCGs.

round and structureless (Fig. 4b). Both types were observed in experimental and control follicles and in all regions of the follicle. Both types were counted together, and the results are shown in Table 1. The degree of significance between control and experimental follicles was determined by Student's *t*-test and graphs plotted from Table 1 are shown in Fig. 5.

Normal control follicles

The numbers of MCGs present in regions A, B, C and D were similar (Table 1). No significant

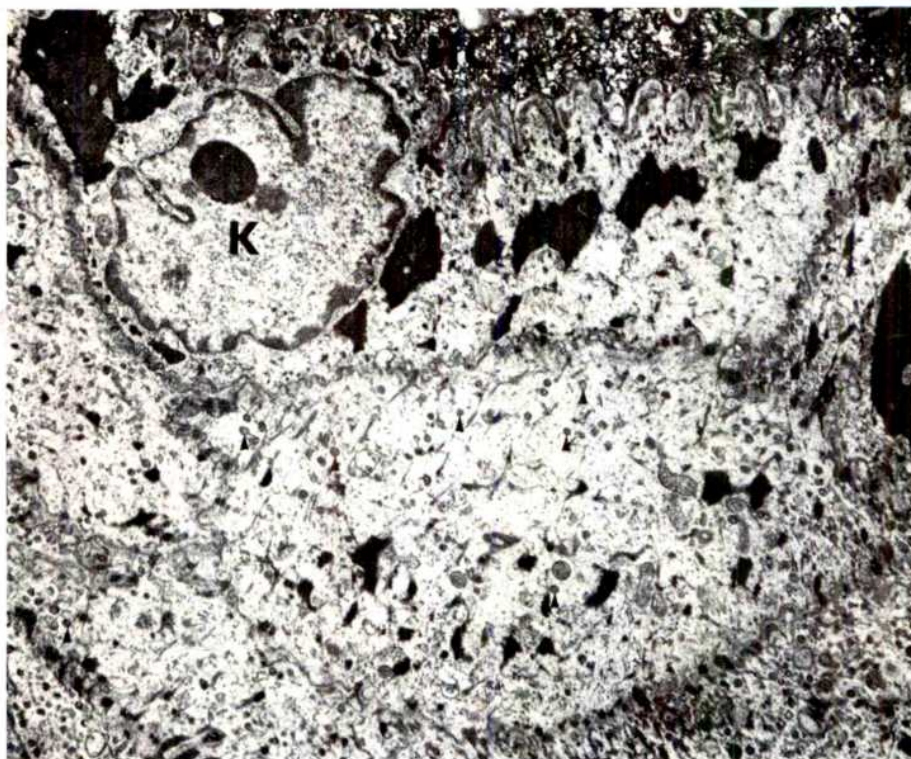


FIGURE 3. Part of the stratum granulosum, showing the greater numbers of MCGs in the lower keratinocytes. Legend as for Fig. 2 ($\times 7500$).

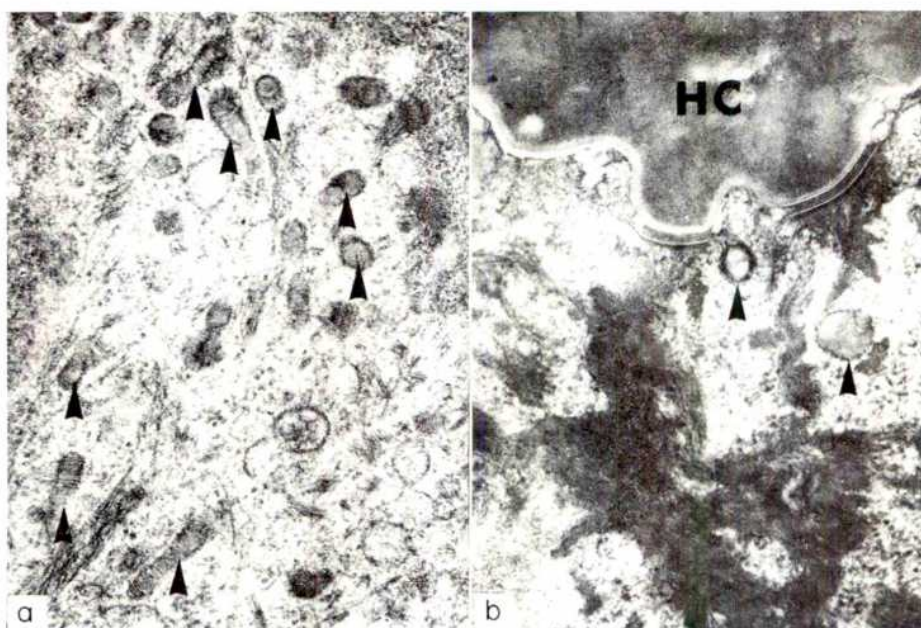


FIGURE 4. (a) MCGs with the typical lamellar structure ($\times 40,000$). (b) Oval and round MCGs without a lamellar structure ($\times 50,000$).

TABLE 1. Number of MCGs present in an area $12 \times 10 \mu\text{m}$ from various regions of control and experimental follicles (mean $\pm$ standard error). Five follicles were sampled in each control group and 10 follicles were sampled in each experimental group

Region of follicle	2 days		4 days		6 days		9 days		14 days		21 days	
	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.
A	147 $\pm$ 5.3	103 $\pm$ 5.2	136 $\pm$ 4.6	79 $\pm$ 4.7	128 $\pm$ 5.2	74 $\pm$ 6.8	125 $\pm$ 5.6	84 $\pm$ 7.4	127 $\pm$ 10.6	81 $\pm$ 3.7	122 $\pm$ 7.2	79 $\pm$ 3.9
B	134 $\pm$ 6.2	104 $\pm$ 5.7	144 $\pm$ 7.5	87 $\pm$ 5.4	129 $\pm$ 6.2	82 $\pm$ 6.7	124 $\pm$ 6.9	79 $\pm$ 6.3	123 $\pm$ 7.5	79 $\pm$ 4.2	122 $\pm$ 8.7	74 $\pm$ 5.1
C	140 $\pm$ 7.6	133 $\pm$ 7.7	155 $\pm$ 6.1	110 $\pm$ 8.2	142 $\pm$ 6.4	87 $\pm$ 5.5	129 $\pm$ 14.8	77 $\pm$ 3.4	125 $\pm$ 7.4	77 $\pm$ 3.2	132 $\pm$ 8.3	68 $\pm$ 4.1
D	140 $\pm$ 4.9	129 $\pm$ 5.7	136 $\pm$ 7.6	105 $\pm$ 4.7	144 $\pm$ 7.2	82 $\pm$ 4.6	136 $\pm$ 10.7	86 $\pm$ 4.4	121 $\pm$ 11.5	78 $\pm$ 5.1	129 $\pm$ 5.2	69 $\pm$ 3.5
E	100 $\pm$ 4.3	91 $\pm$ 11.0	96 $\pm$ 4.4	97 $\pm$ 9.4	97 $\pm$ 4.7	82 $\pm$ 6.7	93 $\pm$ 7.1	90 $\pm$ 5.8	95 $\pm$ 5.4	83 $\pm$ 6.1	104 $\pm$ 7.8	79 $\pm$ 5.3

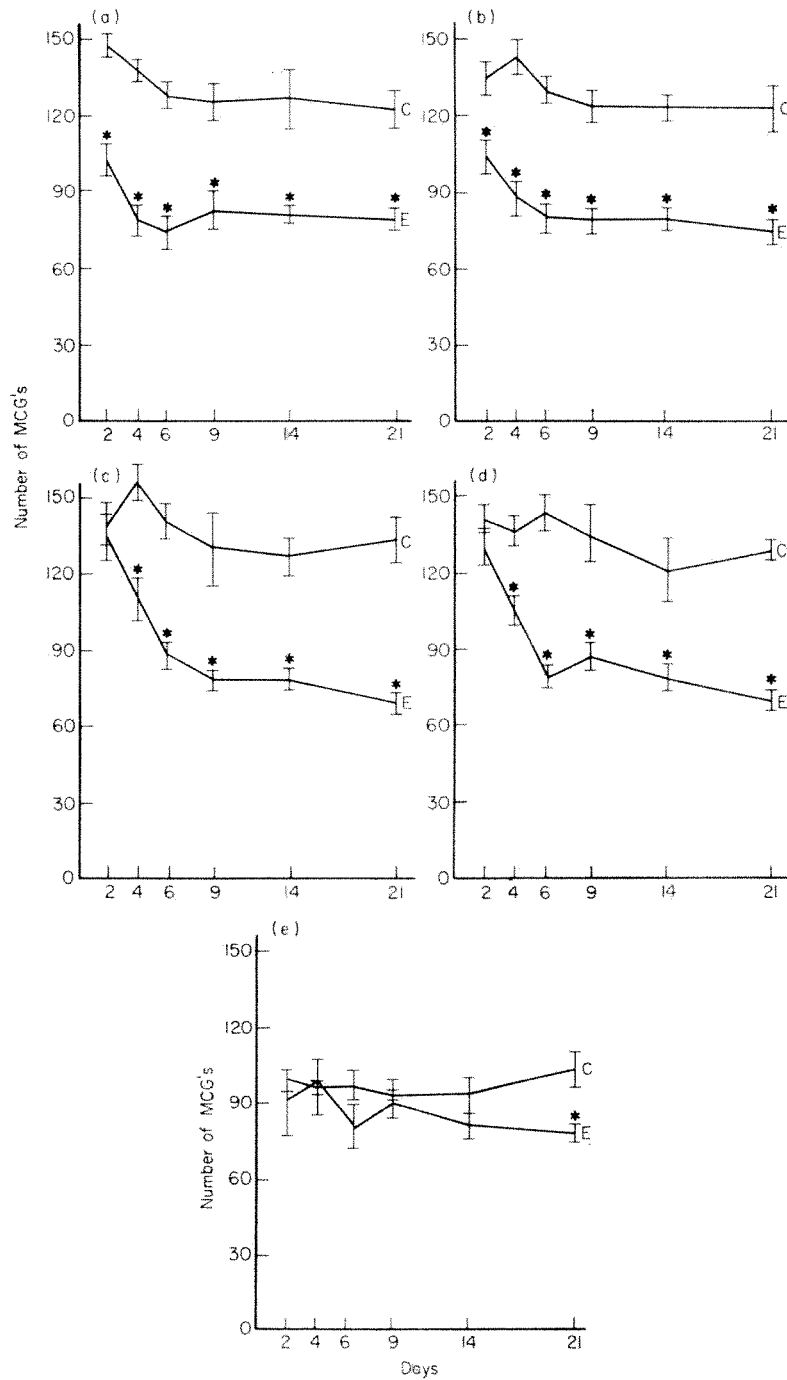


FIGURE 5. Number of MCG's present in various regions of follicles, plotted from Table 1. (a) Region A, (b) region B, (c) region C, (d) region D, (e) region E of follicle. C, control, treated with paraffin oil; E, experimental, treated with 50% oleic acid in paraffin oil; *, $P < 0.01$.

difference was found between them. However, there was a tendency for the numbers to fall slightly during the treatment with paraffin oil (see graphs).

In region E, the MCG count was lower, and it remained fairly constant throughout the treatment with paraffin oil.

Experimental follicles

After 2 days' treatment with 50% oleic acid in paraffin oil, the MCG count decreased significantly in regions A and B (Table 1 and Fig. 5a and b). At this stage, these regions underwent cohesive hyperkeratinization.

Regions C, D and E showed no significant change in the numbers of MCGs (Fig. 5c, d and e). Structurally these regions appeared normal.

On the 4th day, there was a further significant fall of MCGs in regions A and B. At the same time, the numbers of MCGs in regions C and D also began to decrease. In these regions cohesive hyperkeratinization was evident, and the horny cells began to stick together to form a comedo.

After the 6th day, the MCGs' count in regions A and B remained fairly constant. However, there was a further significant drop in the numbers of MCGs in regions C and D, which were now similar in numbers to regions A and B.

Treatment with the comedogenic agent for 9, 14 and 21 days produced no further significant decrease in the numbers of MCGs in the above regions. Throughout the period of treatment, the individual sebaceous ducts showed no significant change, apart from a slight drop in the numbers of MCGs on the 21st day. No obvious structural changes were observed in this region. However, sometimes 1 or 2 cohesive horny cells could be seen in the individual sebaceous ducts, but this was rare.

DISCUSSION

The results show that there is a significant decrease of about 40% in the numbers of MCGs during comedo formation. They agree with the observations of Knutson (1974) on sebaceous follicles of humans with acne.

The decrease in the numbers of MCGs appears to parallel the cohesive hyperkeratinization process. Since cohesive hyperkeratinization takes place at the epidermis and mouth of the follicle first, these are the regions where a fall in the numbers of MCGs is initially found. The follicular canal and common sebaceous duct appear to be affected later (4 days) and this is probably the result of the delayed diffusion of the comedogenic agent into the follicular canal. The individual sebaceous ducts are normally unaffected by the comedogenic agent and consequently the MCGs' numbers were also unaffected.

It appears that the MCGs play an important role in the desquamating process, and it is tempting to postulate that they bring about the disintegration of desmosomes and tight junctions between keratinocytes and horny cells to prepare them for separation (Frenk & Tapernoux, 1976). They probably act as extracellular enzymes (Wolff & Holubar, 1967; Lazarus, Hatcher & Levine, 1975), which break down the intercellular cement substance (Kligman, 1974) between the keratinocytes and horny cells. However, Hashimoto (1971) believes they have a cementing function. In his study of the skin of the toe and nail, he found that the horny layers were firmly attached to each other and showed no signs of degenerating or desquamating although MCGs were abundant. But, as Knutson argues, in comedogenesis their numbers should increase, not decrease as found in this study, if they have a cementing function.

It is possible that two types of MCGs exist, and this would explain their apparently different

functions in different epithelia. Two structurally different types of MCGs were observed in this study: one which was oval or round and structureless and could represent the 'cementsome' (Hashimoto, 1971) and the other with the typical lamellar structure which could contain hydrolytic enzymes (Wilgram & Weinstock, 1970). However, their true chemical nature is debatable (Montagna & Parakkal, 1974).

The close relationship between desquamation and MCGs is further suggested by study of other skin diseases. In a condition described by Frenk & Tapernoux (1976) called hyperkeratosis lenticularis perstans (Flegel), the epidermis is very cohesive (150-300 cell layers) and the horny cells pile up to form a horny papule. The authors reported that there were no or very few MCGs present in the keratinocytes of the epidermis.

Anton-Lamprecht (1974), in a quantitative study of X-linked recessive ichthyosis, observed a decrease in the numbers of MCGs. The stratum corneum appeared thickened and compact with a delayed disintegration of desmosomes and desmosomal bodies, occurring in foci. This may explain the relatively gross scales found in the disease, as the horny cells have become more cohesive.

On the other hand, the present author has found a significant increase in the numbers of MCGs in comedones treated with 0.025% vitamin A acid (unpublished work). This chemical is commonly used in the topical treatment of acne. It also produced hyperkeratinization, and this, combined with the increased numbers of MCGs, probably results in non-cohesive hyperkeratinization, which tends to loosen the comedo and push it out of the follicle (Kligman, Fulton & Plewig, 1969).

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REFERENCES

- ANTON-LAMPRECHT, I. (1974) Zur Ultrastruktur hereditärer Verhornungsstörungen. IV. X-chromosomal-recessive ichthyosis. *Archiv für Dermatologische Forschung*, **248**, 361.
- FRENK, E. & TAPERNOUX, B. (1976) Hyperkeratosis lenticularis perstans (Flegel). A biological model for keratinisation occurring in the absence of Odland bodies? *Dermatologica*, **153**, 253.
- HASHIMOTO, K. (1971) Cementsome, a new interpretation of the membrane coating granule. *Archiv für Dermatologische Forschung*, **240**, 349.
- KLIGMAN, A.M. (1974) An overview of acne. *Journal of Investigative Dermatology*, **62**, 268.
- KLIGMAN, A.M., FULTON, J.E. Jr & PLEWIG, G. (1969) Topical vitamin A acid in acne vulgaris. *Archives of Dermatology*, **99**, 469.
- KNUTSON, D.D. (1974) Ultrastructural observations in acne vulgaris, the normal sebaceous follicle and acne lesions. *Journal of Investigative Dermatology*, **62**, 288.
- LAZARUS, G.S., HATCHER, V.B. & LEVINE, N. (1975) Lysosomes and the skin. *Journal of Investigative Dermatology*, **65**, 259.
- MONTAGNA, W. & PARAKKAL, P.F. (1974) *The Structure and Function of Skin*, 3rd edn. Academic Press, New York and London.
- ODLAND, G.F. (1960) A submicroscopic granular component in human epidermis. *Journal of Investigative Dermatology*, **34**, 11.
- WOLFF, K. & HOLUBAR, K. (1967) Odland-Körper (membrane coating granules, Keratinosomen) abs epidermale Lysosomen. *Archiv für klinische und experimentelle Dermatologie*, **231**, 1.
- WILGRAM, G.F. & WEINSTOCK, M. (1970) Fine structural observations on the formation and enzymatic activity of keratinosomes in the mouse tongue filiform papillae. *Journal of Ultrastructural Research*, **30**, 262.
- WOO-SAM, P.C. (1977) Cohesion of horny cells during comedo formation. An electron microscope study. *British Journal of Dermatology*, **97**, 609.

Leukoderma in the nude C57BL/6 mouse

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SUMMARY

The *nu* gene bred into mice causes the animals to be athymic and nude, that is their hairs do not project past the follicular orifice making them appear to be hairless. The gene has been bred into C57BL/6 mice. C57BL/6 mice which are homozygous for the gene are runted and nude and develop leukoderma around 6 weeks of age. The leukoderma is caused by shedding of short stubby, barely visible black hairs, probably a normal hair cycle. This type of leukoderma is not vitiligo, a disease which causes the skin to turn white because of destruction of the melanocytes in the epidermis or hair bulbs.

The term leukoderma describes any skin which is abnormally hypopigmented or depigmented. Vitiligo is one form of leukoderma. It is a specific disease syndrome caused by destruction of the pigment cells of the epidermis or hair bulb which causes patches of skin and sometimes also the hairs to become white. The processes which destroy the pigment cells remain unidentified. A potential model for the study of vitiligo, the C57BL/6 mouse, has recently been described (Thivolet, 1973, 1975). C57BL/6 mice have a jet black pelage. By special breeding, the *nu* gene which causes the classic nude, athymic mice has been bred into the C57BL/6 strain of mice. The C57BL/6 mice, homozygous for the *nu* gene, are reported to be athymic and nude. The mice develop leukoderma spontaneously. The depigmented skin and hair bulbs were said to be totally depleted of melanocytes (Thivolet, 1973, 1975). We have had an opportunity to examine the C57BL/6 mice homozygous *nu/nu*. In this paper we describe our findings and document that the leukoderma is due to loss of short, stubby hairs, not destruction of the epidermal or hair bulb melanocytes. We believe leukoderma caused by hair loss should not be labelled vitiligo.

MATERIALS AND METHODS

Nude C57BL/6 mice. Five breeding pairs of C57BL/6J1cr/*nu* mice heterozygous for the *nu* gene were

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obtained from the Fox Chase Cancer Center, Philadelphia, Pa. Each pair was marked and kept together until the female was pregnant. For subsequent breedings the same pairs of males and females were re-mated. All litter mates were observed for 3 months. Nude mice homozygous for the *nu* gene were readily recognizable because of runting and absence of a normal pelage. The skin and hairs of these mice were examined carefully with a magnifying glass. Photographs of nude mice were obtained periodically.

Histological examination. Biopsies of the skin extending across the border between the pigmented areas were obtained surgically. The skin sections were stained with standard haematoxylin and eosin, with silver stains for melanin, or with L-dopa (Lerner, 1973) for tyrosinase activity.

Neonatal thymectomy. Newborn C57BL/6 or C3H/HeJ (Jackson Laboratory, Bar Harbor, Maine), ranging in age from a few hours up to 7 days, were attached to a board with rubber bands. A tiny excision was made in the sternum and the thymus removed with suction. Mortality rates were approximately 80%. Successfully thymectomized mice were runted and sickly. Post-mortem examination of dead mice indicated that at least half of those which lived had a total removal of their thymus. Many neonatally thymectomized mice received twice weekly injections of antilymphocyte serum (ALS) and/or antithymocyte serum (ATS) (Microbiological Associates, Bethesda, Md.) to insure a more total depletion of circulating lymphocytes. In a few experiments the pregnant mothers were injected intravenously with 0.5 ml of antithymocyte serum on two occasions prior to delivery of the litter. These neonates were subsequently thymectomized.

RESULTS

Gross examination. At birth all littermates are pink and without pigment or fur. Within a week the skin of the nudes and normals begins to turn black (Fig. 1a). Vibrissae are present on both types of mice. Normal mice and those heterozygous for the *nu* gene develop a typical pelage and are indistinguishable from each other. The nudes remain runted but exhibit short broken hairs projecting from the skin which are visible on careful inspection or with a hand lens. The vibrissae in nude animals are shorter than normal. Around 6 weeks of age white spots begin appearing around the eyes and over the dorsum of the skull and on the face of the nude mice (Fig. 1b). The depigmented spots spread to cover much of the body. Hair bristles are visible projecting from the black skin but not from the depigmented areas (Fig. 2). Some of the depigmented mice regained their black skin colour over a 4-6 week period (Fig. 1c).

Microscopic examination. Hair follicles are present in the black and white skin (Fig. 3). But the follicles in the black skin contain black hair shafts which appear coiled and trapped in the dermal portion of the follicle. Follicles in the white skin are empty. Dopa stains reveal a few lightly stained dendritic pigment cells in the epidermis of both the pigmented and depigmented areas (Fig. 4).

Neonatal thymectomy. Thymectomy of 1-2-day-old mice with or without injections of ALS or ATS produced a slight colour change in the pelage of a few C3H/HeJ mice. The hair was lighter and had a reddish hue. The finding was not consistent nor easily reproducible. No thymectomized mice were nude or had abnormal hair shafts. Plucking of hairs from the thymectomized mice produced no change in the colour of the new pelage. The skin underlying the fur of normal or thymectomized mice was typically white.

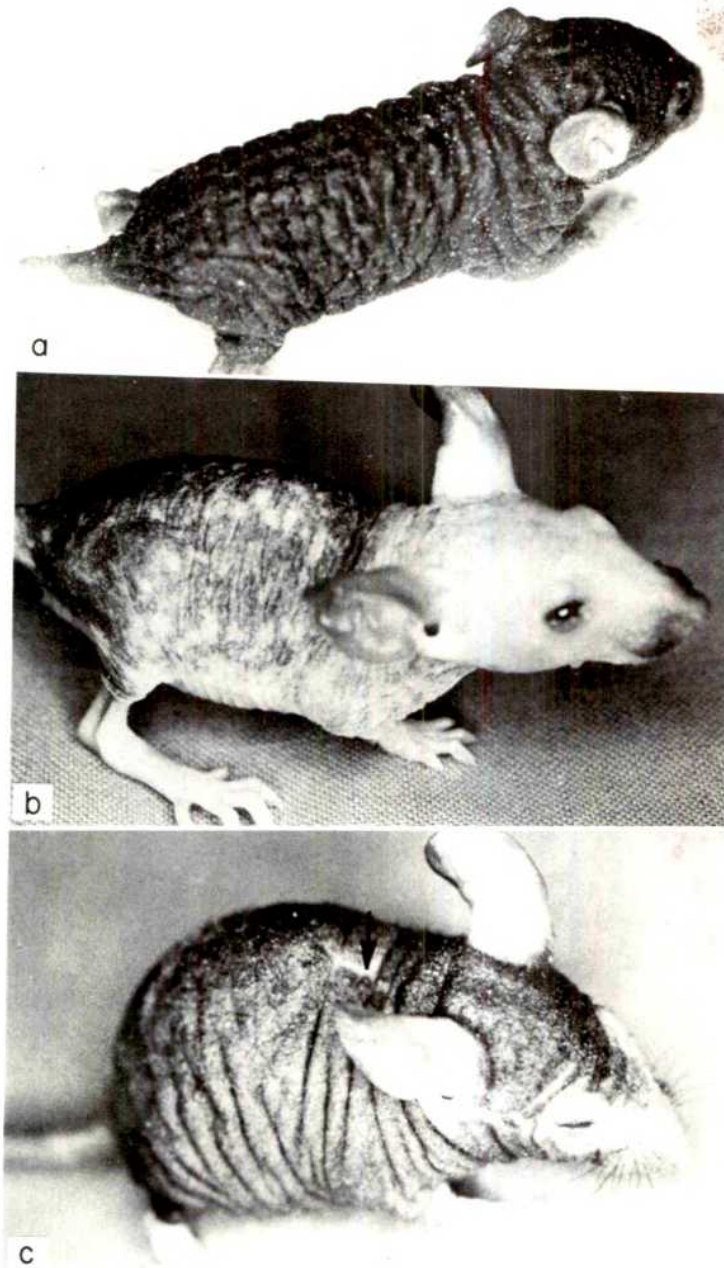


FIGURE 1. (a) Four-week-old nude C57BL/6 mouse showing skin which appears black. (b) Twelve-week-old nude C57BL/6 mouse showing extensive loss of colour especially from around the eyes and from the dorsum of the head. (c) Eighteen-week-old mouse which has regained its original black colour. Note the scar (arrow) caused by a biopsy taken across the border of a previously depigmented area. Hair bristles on the skin are visible on close inspection.



FIGURE 2. Close-up view of a dark area of skin of a nude C57BL/6 mouse showing the bristles of hair in the dark areas. No hairs are visible in the white areas.

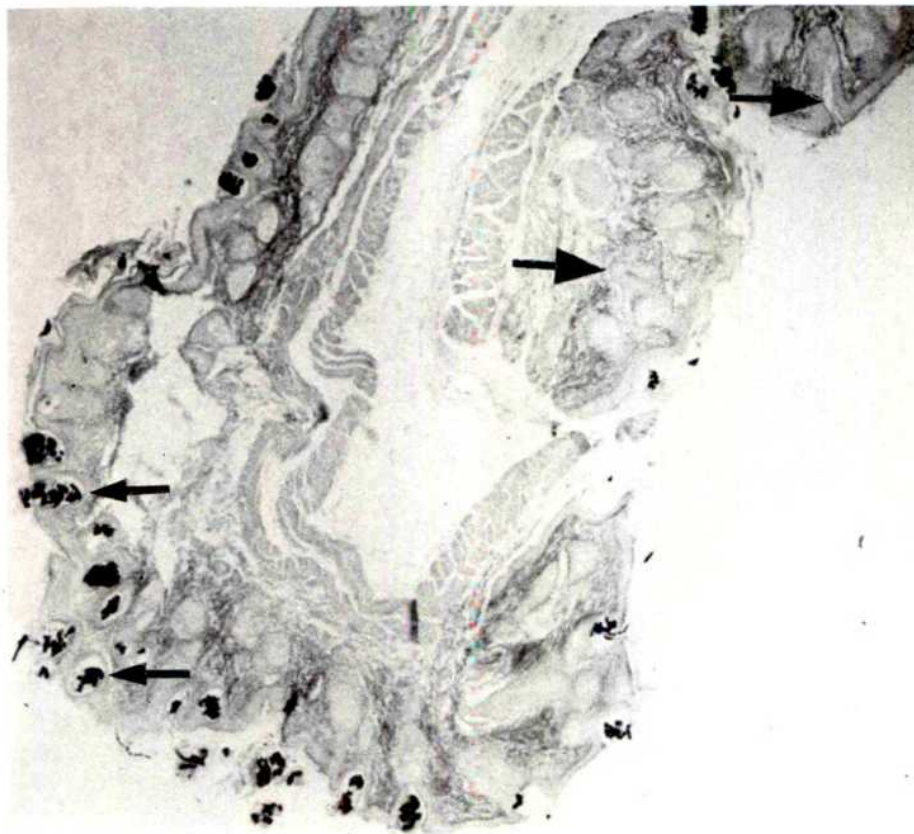


FIGURE 3. Low power view of a skin biopsy showing hair follicles in both pigmented (small arrows) and non-pigmented (large arrows) areas. The epidermis on the right side was white and the follicles lack hair shafts. The follicles on the left, from dark skin, contain coiled hair shafts which are about to be shed (H & E, $\times 40$).

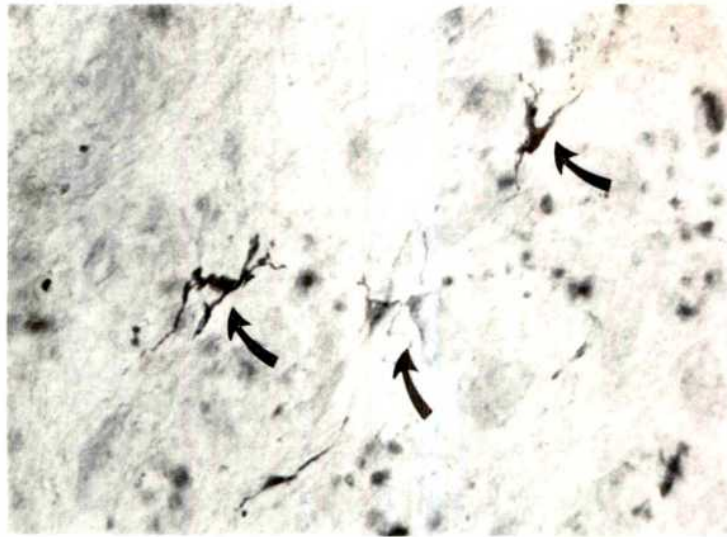


FIGURE 4. High power view of depigmented skin showing presence of tyrosinase positive melanocytes (arrows) in the epidermis (H & E, $\times 400$).

DISCUSSION

The nude C57BL/6 mice developed darkly pigmented skin after birth. Around 6 weeks of age, waves of depigmentation beginning around the eyes and over the dorsum of the head spread over much of the integument. In normal C57BL/6 mice, thymectomy at birth, a potent technique of immunosuppression stimulating the *nu* gene, produced a slightly hypopigmented pelage as has been previously reported (Thivolet, 1973, 1975).

The epidermis of mice normally has very few melanocytes (Wolfe, 1975). Most pigment cells in the skin of the mouse are within the hair bulbs. Plucking the hair off the C57BL/6 mouse reveals white skin. When the plucked hair begins to regrow, i.e. when the hair is forming within the follicles, skin temporarily appears black. After the black hairs extend beyond the follicles the skin again appears white. Nude mice are not hairless (Rigden, 1974). Hairs are present but break off outside the hair follicle giving the animal a hairless appearance (Rigden, 1974; Eaton, 1976). The short broken hairs, which are black in the nude C57BL/6 mice, cause skin of the 7–10-day-old mice merely to appear black. As the hairs spontaneously fall out due to normal hair cycles, the skin appears to have turned white. Hair cycles in the nude mouse typically begin around the eyes and progress in an anteroposterior direction. Hair growth cycles in the homozygous *nu/nu* C57BL/6 mouse are more rapid than in normal mice (Eaton, 1976).

Epidermal and hair bulb melanocytes are demonstrable in the black and white skin of these animals. Their number and distribution in response to dopa stains are typical for all mice. These nude mice do develop a variety of leukoderma. It is due, however, to the loss of terminal hairs and not a specific destruction of melanocytes.

Neonatal thymectomy produces some slight decrease in the colour of the pelage in a few C3H/HeJ or C57BL/6 mice. The animals successfully thymectomized are runted and sickly. Possibly the dilution of pigment in the pelage of these animals is similar to the 'flag sign' observed in human subjects with severe nutritional deficiency such as kwashiorkor.

The distinction between vitiligo and other forms of leukoderma in animal models as well as in human subjects is important and worth while. Precision in communication depends upon accurate descriptions and definitions of terms. Secondly, the nosology of pigmentary disorders influences the thinking of the scientific community about disease processes. Vitiligo is a destructive disease of melanocytes associated with certain autoimmune diseases such as lymphomas, melanomas, thyroiditis and even alopecia areata (Lerner, 1978). The associations of vitiligo with other diseases seem to be important clues to the aetiology and possibly treatment of vitiligo. For animal models to be applicable to the study of the human disease, the disease exhibited by the animal should be similar to that expressed by the human subjects.

REFERENCES

- EATON, G.J. (1976) Hair growth cycles and wave patterns in nude mice. *Transplantation*, **22**, 217.
- LERNER, A.B. & HENDE, J. (1973) Rapid histochemical test for mammalian tyrosinase. *Journal of Investigative Dermatology*, **60**, 16.
- LERNER, A.B. & NORDLUND, J.J. (1978) Vitiligo: What is it? Is it Important? *Journal of the American Medical Association*, in press.
- RIGDON, R.H. (1974) Histologic study of the skin of congenitally athymic 'nude' mice. *Texas Reports on Biology and Medicine*, **32**, 711.
- THIVOLET, J., ORTONNE, J.P., MONIER, J.C., PERROT, H. & SCHMITT, D. (1973) Depigmentation of vitiliginous appearance in nude mice. In: *Proceedings of the First International Workshop on Nude Mice* (Ed. by J. Rygaard and C.Povlsen), p. 227. Gustav Fischer Verlag, Stuttgart.
- THIVOLET, J., ORTONNE, J.P., MONIER, J.C. & PERROT, H. (1975) Berichte und Briefe an die Herausgeber—Vitiligo, Thymusdefekt und Auto-immunität—eine Hypothetische Pathogenese. *Der Hautarzt*, **26**, 50.
- WOLFE, G. & COLEMAN, D.L. (1975) Pigmentation. In: *Biology of the Laboratory Mouse* (Ed. by E.Green), 2nd edn, p. 405. Dover Publications, New York.

Complement components in blood serum and suction blister fluid in erythropoietic protoporphyria

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SUMMARY

Complement factors C3c, C4, factor B, C3d and the immunoglobulin IgA were determined in suction blister fluid of patients with erythropoietic protoporphyria and in normal controls. The levels increased when the skin of the patients had been previously irradiated with 'white light', but not with Kromayer radiation, and were interpreted as being the result of increased vasopermeability.

The skin of patients who are exposed to visible light and suffer from erythropoietic protoporphyria (EPP) may develop delayed oedema and purpura (Magnus, 1968), reactions which we have studied by light and electron microscopic examination of experimentally irradiated non-light exposed skin with glass-filtered mercury arc radiation (so-called 'white light', Rottier, 1969) or violet light. Deposition of complement (C) factors (C1q, C3c, C3d, C4, C5), immunoglobulins (mainly IgM) and fibrin (and/or fibrinogen) was observed within the confines of the superficial capillary walls, together with large perivascular fibrinous exudates (Baart de la Faille, 1975).

Fluid from suction blisters in the present work has been analysed for C components and IgA, after irradiation with 'white light'.

MATERIALS AND METHODS

The experiments concerned 2 patients with EPP (a woman aged 31 and a man aged 28). 'White light' was produced with an apparatus consisting of a super high pressure mercury arc (Philips SP 500 W) and interference filters in a water-cooled housing (Rottier, 1969). Care was taken to keep the temperature of the skin below 42°C during the irradiation. The irradiation dose used was approximately 150 J/cm². Control specimens of suction fluid obtained from water-cooled Kromayer irradiated skin (10 MED) and non-irradiated skin were examined. Suction blisters were also provoked on the non-

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irradiated skin of 2 healthy volunteers (a man, aged 26 and another, aged 27) whose reaction to light was normal.

The suction procedure was undertaken within 1 h after irradiation had been completed. The tests were performed on the dorsal side of the forearm. Blisters were produced with Suction cups (Van der Leun *et al.*, 1974) and an additional apparatus which provided possibilities for automatic control of suction pressure and skin temperature (Beerens, 1977), in our experiments at $p = 200$ mmHg and $t = 41^{\circ}\text{C}$ respectively. The orifice of the suction cup was 15 mm in diameter. Blister formation started approximately 1 h after applying suction, but it took 2–3 h for large bullae, covering the entire suction area, to develop. When suction was terminated, the cups were removed and blister fluid was collected with a plastic syringe fitted with a 25 gauge needle and the fluid was stored at -70°C . The volume of fluid varied from 0.1 to 0.3 ml.

We determined complement components C3c, C4 and factor B (of the alternative complement pathway) and the immunoglobulin IgA in aliquots of undiluted blister fluid by simple radioimmuno-diffusion, and used commercial antiserum-containing agarose plates (Partigen, Behringwerke, Germany). C3-fragment C3d was estimated by electroimmunodiffusion according to Laurell (1966); 5 μl of undiluted blister fluid were electrophoresed into agarose gel containing rabbit antihuman C3d (Red Cross Blood Transfusion Service, Amsterdam) in 1:20 dilution. Factor C9 in blood serum was similarly determined by means of electroimmunodiffusion; CH₅₀ values for the total amount of haemolytic complement in serum were determined, and read from Van Krogh plots.

RESULTS

Table 1 shows the levels of some complement components and of IgA in suction blister fluid of EPP patients before and after irradiation. The amounts of C3c, factor B, C4, and IgA in blister fluid of the untreated or Kromayer irradiated skin averaged 25% of those found in normal sera or in the blood of asymptomatic EPP patients (Table 2). The concentration of split product C3d was about half that found in a pool of normal sera. In both patients with EPP, all complement values increased, as did the IgA, when blister fluid had been obtained after 'white light' irradiation of the suction area.

TABLE 1. Some complement components and IgA in suction blister fluid of irradiated and non-irradiated skin of patients with EPP and of healthy volunteers

Subject	Treatment	C3c	C3d	C4	Factor B	IgA
EPP, female, age 31	Non-irradiated	29	76	11.4	6	68
	Kromayer	29	65	11.4	6	60
	'White light'	44	103	21.0	11.6	110
EPP, male, age 28	Non-irradiated	15	42	5.4	4.9	34
	Kromayer	19	68	8.0	5.3	52
	'White light'	40	92	17.2	10.4	98
Control, male, age 26	Non-irradiated	22	61	5.4	7.0	72
Control, male, age 27	Non-irradiated	16	47	5.4	4.3	10

Values in mg/100 ml, C3d in percent of normal serum pool.

Insufficient blister fluid was available to determine albumin as a parameter for serum leakage.

TABLE 2. Some complement components in the blood serum of a group of patients with EPP, showing no signs of an acute response to sunlight at the time of blood sampling

Protein component(s)	EPP			Normal controls: normal range
	N	Mean $\pm$ s.d.	Range	
CH ₅₀ -value	14	78 $\pm$ 12	62-95	60-100
C3c (mg/100 ml)	14	96 $\pm$ 15	68-122	55-100
C4 (mg/100 ml)	14	39 $\pm$ 9	22-55	20-45
C9 (% of normal)	14	141 $\pm$ 36	87-204	80-150
Factor B (mg/100 ml)		N.D.		15-35

Arithmetic mean $\pm$ s.d. indicated. Normal range as established in our laboratory
N = number of patients.

We were unable to detect significant differences between the serum levels of complement factors of EPP patients and those established for normal volunteers in our laboratory (Berrens *et al.*, 1976).

DISCUSSION

The quantity of serum components in suction blister fluid increased after the skin had been previously irradiated with 'white light'. Kromayer radiation did not have this effect and the values were approximately equal to those of unirradiated skin of patients and healthy persons (Table 1). These results are compatible with previous findings of damage done by 'white light' to the superficial capillaries of the dermis of EPP patients (Baart de la Faille, 1975).

We tentatively conclude that 'white light' induces an increased permeability of the capillary walls, leading to leakage of serum protein components into the surrounding tissue and afterwards into the blister fluid. Our methods do not permit conclusions as to the activation of the complement system by the irradiation.

Schmid (1970) found IgG, IgM and albumin in friction blister fluid, averaging 10% of the corresponding serum levels. Jordon *et al.* (1978) reported functional C components in suction blister fluid of pemphigus patients at 55-74% the normal serum values. These results, and the absence of factor B from pemphigus blister fluid, may depend on the nature of the blistering disease or the technique of experimental blister induction.

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REFERENCES

- BAART DE LA FAILLE, H. (1975) *Erythropoietic protoporphyria: a photodermatosis. Microscopical and electron microscopical investigations of irradiated skin and a clinical study*. Doctoral thesis, Utrecht University. (Oosthoek, Scheltema & Holkema, Utrecht, 1975).
- BEERENS, E.G.J. (1977) *Dermal-epidermal adherence. A study on repair after separation effected by suction blistering*. Doctoral thesis, Utrecht University. (Elinkwijk, Utrecht, 1977).
- BERRENS, L., JANKOWSKI, E. & JANKOWSKI-BERNTSEN, I. (1976) Complement component profiles in urticaria, dermatitis herpetiformis and alopecia areata. *British Journal of Dermatology*, **95**, 145.

- JORDON, R.E., DAY, N.K., LUCKASEN, J.R. & GOOD, R.A. (1973) Complement activation in pemphigus vulgaris blister fluid. *Clinical and Experimental Immunology*, **15**, 53.
- LAURELL, C.B. (1966) Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analytical Biochemistry*, **15**, 45.
- LEUN, J.C. VAN DER, LOWE, L.B., JR & BEERENS, E.G.J. (1974) The influence of skin temperature on dermal-epidermal adherence: evidence compatible with a highly viscous bond. *Journal of Investigative Dermatology*, **62**, 42.
- MAGNUS, I.A. (1968) The cutaneous porphyrias. *Seminars in Hematology*, **5**, 380.
- ROTTIER, P.B. (1969) The ratio of the 260/300 nm MED's in the judgement of skin sensitivity to short wave ultraviolet radiation. In: *The Biological Effects of Ultraviolet Radiation (with Emphasis on the Skin)* (Ed. by F. Urbach), pp. 187. Pergamon Press, Oxford.
- SCHMID, P. (1970) Quantitation of specific proteins in blister fluid. *Journal of Investigative Dermatology*, **55**, 244.

Genital warts: incidence of associated genital infections

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SUMMARY

Two hundred and seventy-eight men and 200 women who presented to a Special Treatment Clinic with genital warts were screened for accompanying genital infections. One hundred and twenty-nine of 212 presentations (61%) in women compared to 98 of 303 presentations (32%) in men were accompanied by another genital infection ($P < 0.001$). Recurrent presentation with warts was commoner in men ($P < 0.001$). In both sexes recurrences were less likely to be accompanied by another genital infection. ($P < 0.002$).

Yeasts (25%), *C. vaginale* (21%), *N. gonorrhoea* (12%) and *T. vaginalis* (12%) were the commonest pathogens in women. Non-specific genital infection (17%) and gonorrhoea (10%) were the commonest accompanying infections in men.

The identification and treatment of these infections, especially in women, is important for the rapid eradication of warts, for, by increasing genital moisture they can create and maintain a favourable environment for wart proliferation. In addition to screening all patients with warts for other sexually transmitted diseases, women should be screened for yeasts and *C. vaginale*.

Patients with genital warts may present to the genito-urinary physician or surgeon, the gynaecologist or dermatologist. The infective agent, a papilloma virus (Oriel & Almeida, 1970), is often sexually transmitted (Theokharov, 1969). Genital warts may be associated with other sexually transmitted infections (Morton, 1975).

Although debility and serious complications are rare, warts distress patients and may necessitate frequent attendances for treatment. In the five years from 1971, the number of wart infections presenting to Special Clinics in England increased by 60%. They are therefore of importance as a cause of increasing clinic work load, and as a marker for the presence of other sexually transmitted diseases. This retrospective study of all patients presenting to the Sheffield Special Treatment Clinic from 1 January until 31 December 1976, seeks to determine the frequency of genital infections accompanying warts in both sexes and to assess their significance.

PATIENTS AND METHODS

Two hundred and seventy-eight men and 200 women presented with warts during the study period. Each was routinely examined for other genital infections. Specimens were prepared in women from the following sites: Urethra and cervix—swabs for Gram stained smears and inoculation of selective media for gonococcal culture; vagina—a wet film preparation; swabs for *Corynebacterium vaginale* culture; Gram stained smears in those with symptomatic vaginal discharge or vulval irritation.

In men with urethral discharge, a wet film preparation, a Gram stained smear and gonococcal culture were prepared. Rectal smears and cultures were taken in all passive homosexuals and a sub-preputial smear made in those with balanitis. Any genital ulcer was repeatedly examined for *Treponema pallidum* and swabs taken for herpes virus culture.

All patients had serological testing for syphilis on their first attendance, repeated if possible after 6 weeks and 3 months.

The following diagnostic criteria were used:

Gonorrhoea—positive microscopic findings confirmed by positive culture;

Trichomoniasis—identification of the parasite on the wet film preparation;

Yeast infection—presence of pseudohyphae and spores on microscopy;

C. vaginale infection and genital herpes—positive culture of the micro-organism.

Non-specific genital infection was diagnosed in men with purulent or mucopurulent urethral discharge, in women with clinical cervicitis with purulent cervical secretion, where no other pathogen was demonstrated.

Pediculosis pubis, scabies and molluscum contagiosum were diagnosed by their clinical appearances. Each individual was assessed for:

(1) Those genital infections diagnosed within 1 week of first attendance with genital warts.

TABLE 1. Accompanying genital infections diagnosed at time of warts presentation

	Male	Female
Total no. of infections with warts	303	212
No. with an associated genital infection	98 (32.3) ($\chi^2 = 40.0$; $P < 0.001$)	129 (60.8)
No. without an associated genital infection	205 (67.7)	83 (39.2)
Nature of associated infection		
Gonorrhoea	30 (9.9)	26 (12.3)
Non-specific genital infection	51 (16.8)	2 (0.9)
Trichomoniasis	3 (1.0)	25 (11.8)
Yeasts	3 (1.0)	52 (24.5)
Scabies	1 (0.3)	1 (0.5)
Pediculosis pubis	6 (2.0)	3 (1.4)
Herpes genitalis	3 (1.0)	3 (1.4)
Molluscum contagiosum	1 (0.3)	—
Balanitis (non specific)	11 (3.6)	—
<i>Corynebacterium vaginale</i>	—	44 (20.9)

(2) Those genital infections which had been acquired at any time within preceding 12 months requiring treatment.

Statistical comparisons were made by Yates modified χ^2 test. Results are given at one degree of freedom.

RESULTS

An accompanying genital infection was diagnosed in 129 of 212 presentations (61%) in women and 98 of 313 presentations (32%) in men. This highly significant sex difference ($P < 0.001$) was also found in the comparison of numbers of men and women who had acquired another genital infection within the preceding 12 months (Table 2).

The commonest associated infections in both sexes were those which were frequently recurrent, being caused by yeasts (25%) and *C. vaginale* (21%) in women, and non-specific genital infection (17%) in men.

Recurrence of genital warts was commoner in men ($P < 0.001$) (Table 3). Those of either sex who had recurrent warts were less likely to have an accompanying infection than those who presented once ($P < 0.002$). There was, however, no difference in the proportions acquiring other genital infections in the preceding 12 months.

TABLE 2. Total genital infections diagnosed either at presentation or in the 12 months preceding presentation with warts

	Male	Female
No. of patients presenting with warts in study period	278	200
No. presenting with warts on more than one occasion in study period	23 (8.3) ($\chi^2 = 1.46$; $P > 0.1$)	10 (5.0)
No. of patients diagnosed with warts in preceding 12 months	47 (16.9) ($\chi^2 = 9.4$; $P < 0.005$)	14 (7.0)
No. of patients with another genital infection	134 (48.2) ($\chi^2 = 25.0$; $P < 0.001$)	143 (71.5)
No. of patients with no other genital infection	144 (51.8)	57 (28.5)
Nature of infection		
Gonorrhoea	48 (17.3)	38 (19.0)
Non-specific genital infection	78 (28.1)	2 (1.0)
Trichomoniasis	4 (1.4)	32 (16.0)
Yeast	5 (1.8)	70 (35.0)
Scabies	1 (0.4)	1 (0.5)
Pediculosis pubis	7 (2.5)	4 (2.0)
Herpes genitalis	4 (1.4)	3 (1.5)
Molluscum contagiosum	1 (0.4)	—
Balanitis (non specific)	13 (4.7)	—
<i>Corynebacterium vaginale</i>	—	46 (23.0)

TABLE 3. Comparison of those presenting on a single occasion with warts and those with recurrent warts

	Male		Female	
	Single presentation	Recurrent	Single presentation	Recurrent
No. of patients	208 (74.8)	70 (25.2)	176 (88.0)	24 (12.0)
		$(\chi^2 = 12.0; P < 0.001)$		
No. of patients who had another infection at warts presentation or preceding 12 months	102 (49.0)	32 (45.7)	126 (71.6)	17 (70.8)
No. of wart infections in study period	208	95	176	36
No. of wart infections with accompanying infection	80 (38.5)	18 (18.9)	116 (65.9)	13 (36.1)
	$(\chi^2 = 10.5; P < 0.002)$		$(\chi^2 = 9.9; P < 0.002)$	

DISCUSSION

Warmth and persistent moisture are major factors which allow proliferation of genital warts (Oriol, 1971). A suitable minitropical environment is promoted by poor hygiene and the wearing of nylon tights and underwear, especially in the obese. In the uncircumcised male, ideal conditions exist in the sub-preputial sac. Any infection which increases genital secretion will predispose to wart proliferation. Persistence of this additional moisture will increase the difficulty in eradicating warts and prolong treatment duration.

The results suggest that accompanying genital infections are of special importance in women in creating such a favourable environment. Bramley (personal communication) in a study of 522 women presenting to a Sheffield Family Planning Clinic found yeast infection in 58 (11%), *C. vaginale* in 29(6%), *T. vaginalis* in 8 (2%) and *N. gonorrhoeae* in 1.

Other authors have reported on genital infections accompanying warts. The high incidence of yeast infection is supported by Oriol (1971) and of trichomoniasis and gonorrhoea by Fox & Spencer (1977).

A difference in the pathogenicity of the causative micro-organism may explain the marked sex differences in incidence of associated yeast, *C. vaginale* and *T. vaginalis* infection. Oral contraceptive preparations, which were used by 121 females (60.5%) presenting with warts, increase susceptibility to vaginal yeast infection (Catterall, 1971).

The pathogenic significance of *C. vaginale* remains disputed as the organism frequently exists in asymptomatic patients. Gardner & Dukes (1955) found a significant increase in the incidence of *C. vaginale* cultures from women with symptomatic vaginal discharge compared with control groups. Lewis *et al.* (1971) reviewed 1008 patients and concluded that the organism was a definite vaginal pathogen. Our experience is that it is associated with excessive vaginal discharge but signs of vaginitis are often minimal. The possibility of asymptomatic carriage of yeasts and *C. vaginale* in the urethra and subpreputial sac of men with warts requires further investigation.

A diagnosis based on positive identification of a definite pathogen, such as chlamydia, might have given a smaller sex difference in the incidence of nonspecific genital infection.

There may be some selection of patients who attend special treatment clinics with warts. Other doctors tend to refer those with additional symptoms or those who prove resistant to treatment. The open access to Special Clinics allows patients to attend without a referral letter or pre-arranged appointment. Those who have a history of attendance with previous genital infections are more likely to return if new genital symptoms develop.

The long incubation period of the papilloma virus allows infections acquired simultaneously to become symptomatic and require treatment, or spontaneously remit, before warts appear. A high proportion of male gonococcal infections were of more recent acquisition than were their warts. In many asymptomatic patients, however, particularly in women and passive homosexuals, the appearance of genital warts provided the stimulus for the patients' presentation and resulted in the discovery, by routine screening, of other longstanding sexually transmissible disease.

REFERENCES

- BRAMLEY, M. (1978) Personal communication.
- CATTERALL, R.D. (1971) Influence of gestogenic contraceptive pills on vaginal candidosis. *British Journal of Venereal Diseases*, **47**, 45.
- FOX, M. & SPENCER, T.D. (1977) Genital warts. *British Journal of Sexual Medicine*, **13**.
- GARDNER, H.L. & DUKES, C.P. (1955) *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified as non-specific vaginitis. *American Journal of Obstetrics and Gynecology*, **69**, 962.
- LEWIS, J.F. O'BRIEN, S.M., URAL, U.M. & BURKE, R. (1971) *Corynebacterium vaginale* vaginitis. *American Journal of Obstetrics and Gynecology*, **112**, 87.
- MORTON, R.S. (1975) *Recent Advances in Sexually Transmitted Diseases*, Number one (Ed. by R.S. Morton and J.R.W. Harris), pp. 338. Churchill Livingstone, Edinburgh.
- ORIEL, J.D. (1971) The natural history of genital warts. *British Journal of Venereal Diseases*, **47**, 1.
- ORIEL, J.D. & ALMEIDA, J.D. (1970) Demonstration of virus particles in human genital warts. *British Journal of Venereal Diseases*, **46**, 37.
- THEOKHAROV, B.A. (1969) Non gonococcal infections of the female genitalia. *British Journal of Venereal Diseases*, **45**, 334.

Pharmacology and Treatment

A study of the comparative efficacy of diflucortolone valerate 0.3% ointment and clobetasol propionate 0.05% ointment

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SUMMARY

Three hundred and fifty-four patients with symmetrical dermatoses took part in a multicentre, double-blind, half-side study in order to compare the efficacy of a new topical steroid, diflucortolone valerate 0.3% (Nerisone Forte) against that of an established, potent topical steroid, clobetasol propionate 0.05% (Dermovate).

The assessment of overall response, as judged by the physicians' preference for one side or another, showed no difference between the two compounds. However, when the results were examined by separate diagnostic category, the number of preferences was greater for diflucortolone valerate 0.3% in eczema, and for clobetasol propionate 0.05% in psoriasis, although neither of these differences reached levels of statistical significance. The graded assessments of response indicated that both compounds were highly effective, potent, topical steroids. Eighty-one percent of all patients showed marked improvement or healing with diflucortolone valerate 0.3%, and 84% showed marked improvement or healing with clobetasol propionate 0.05%. This difference was not statistically significant. Analysis of response, either by diagnosis or grade of severity, showed no statistically significant differences between the two compounds. No significant differences in the incidence of severity of side-effects were observed.

It was concluded that the two compounds were of equal clinical efficacy.

Diflucortolone valerate is a new topical corticosteroid (see Fig. 1) which has been shown to be very potent in animals (Kapp *et al.*, 1976). In an investigation of 35 patients, using the special technique of the psoriasis plaque test, it was demonstrated that the 0.3% concentration of diflucortolone valerate (Dfv 0.3%) was comparable in efficacy to clobetasol propionate 0.05% (Löffler, 1978). Since clobetasol propionate 0.05% (clobetasol) is considered to be one of the most potent of the topical corticosteroids available (Sparkes & Wilson, 1974; Sneddon, 1976), it was thought advisable to confirm the results of the psoriasis plaque test in a clinical investigation of patients with different corticoid-responsive dermatoses. Thus, to obtain meaningful results, and bearing in mind the variations in

conditions and severity of lesions, it was appreciated that large numbers of patients would be required. In order to compare clobetasol propionate 0.05% ointment with diflucortolone valerate 0.3% ointment, a multicentre study was set up, with patients being investigated using a double-blind half-side comparative design and random allocation of each preparation to either left or right side.

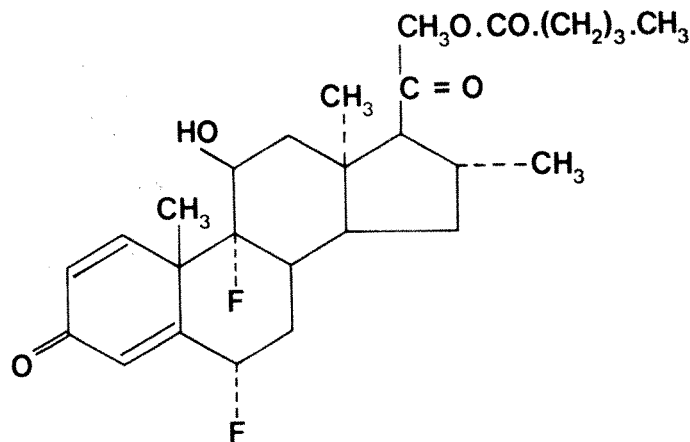


FIGURE 1. Diflucortolone valerate (6a, 9-diflu-11 β -hydroxy-21-valeryloxy-16 α -methyl-1, 4-pregnadiene-3, 20-dione).

PATIENTS AND METHODS

Three hundred and fifty-four patients with symmetrical chronic dermatoses for which potent topical steroid therapy was applicable were enrolled. 219 were male and 132 were female (3 not recorded); the mean age was 44.95 years with a standard deviation of 20.54 years. The diagnosis, degree of severity, duration of the condition and duration of exacerbation were recorded.

The compounds were packed in identical tubes, which were labelled left or right and allocated according to the randomisation schedule. The majority of cases were out-patients and they were instructed to use both preparations equally, applying the ointments thinly and twice daily to the skin of the respective sides. Some of the patients were hospitalized; in these cases the application of each ointment to the correct side was made by the nurse. The maximal dose of each ointment, which was used over the whole treatment period of up to 3 weeks, was 100 g.

Patients were reviewed weekly. At each review, the physicians assessed the efficacy of each preparation by grading the response in relation to the initial condition. This was done on a 5 point scale representing worse, static, slightly improved, markedly improved or healed.

At the end of the treatment period, physicians compared the response of each preparation in terms of degree and rate of healing or side-effects, and indicated a preference for right or left side, where possible.

Statistical analysis was by means of the binomial test, for treatment preferences, at a level where $P = 0.05$, and by the 't'-test for comparing percentages of the efficacy data.

RESULTS

On breaking the treatment code in order to determine which compound had been applied to left or right side in each individual patient, it was apparent that the two compounds had achieved an equal

total number of preferences (see Table 1). Analysis of preferences by diagnosis indicated that in patients with psoriasis, clobetasol had a higher number of preferences (40) than Dfv 0.3% (28), but that in eczematous lesions Dfv 0.3% had a higher number (33) than clobetasol (25). Neither of these two differences reached levels of statistical significance.

TABLE 1. Analysis by diagnosis

	Physician's preference		
	Diffucortolone	Equal	Clobetasol
Psoriasis	28 (21%)	66 (49%)	40 (30%)
Atopic dermatitis	9 (22%)	26 (63%)	6 (15%)
Eczema	33 (22%)	93 (62%)	25 (17%)
Others	8 (29%)	13 (46%)	7 (25%)
Total	78 (22%)	198 (56%)	78 (22%)

The graded assessments of response at the end of the treatment period are shown in Table 2. These assessments were analysed for the total number of patients and also for the separate diagnostic categories. No statistically significant differences were found between the two compounds, either overall or by diagnosis.

Since both Dfv 0.3% and clobetasol are frequently used in severe dermatoses, it was decided to examine the results in order to investigate whether or not the overall results masked a difference between the two compounds according to the severity of the lesion. Table 3 illustrates the graded response of the final condition of the patient's lesion, when analysed according to initial severity, from which it is seen that the results for the two compounds are very similar; the small differences that were found were not statistically significant.

It was nevertheless considered possible that differences might exist between the responses of severe lesions to the compounds according to diagnosis. Analysis of the 111 severe cases by diagnosis was

TABLE 2. Analysis by diagnosis

		Physician's assessment				
		Healed	Marked improvement	Slight improvement	Static	Total
Psoriasis	Diffucortolone	28 (21%)	71 (53%)	33 (25%)	2 (1%)	134
	Clobetasol	29 (22%)	79 (59%)	24 (18%)	2 (1%)	134
Atopic dermatitis	Diffucortolone	14 (34%)	21 (51%)	6 (15%)	0	41
	Clobetasol	13 (32%)	20 (49%)	8 (20%)	0	41
Eczema	Diffucortolone	59 (39%)	69 (46%)	20 (13%)	3 (2%)	151
	Clobetasol	55 (36%)	75 (50%)	16 (11%)	5 (3%)	151
Others	Diffucortolone	7 (25%)	18 (64%)	2	1	28
	Clobetasol	6 (21%)	20 (71%)	1	1	28
Total	Diffucortolone	108 (31%)	179 (51%)	61 (17%)	6 (2%)	354
	Clobetasol	103 (29%)	194 (55%)	49 (14%)	8 (3%)	354

TABLE 3. Analysis by severity

		Physician's assessment				
		Healed	Marked improvement	Slight improvement	Static	Total
Severe	Diffucortolone	29 (26%)	60 (54%)	20 (18%)	2 (2%)	111
	Clobetasol	29 (26%)	63 (57%)	17 (15%)	2 (2%)	111
Moderate	Diffucortolone	68 (31%)	107 (49%)	40 (18%)	4 (2%)	219
	Clobetasol	64 (29%)	119 (54%)	30 (14%)	6 (3%)	219
Mild	Diffucortolone	10 (53%)	8 (42%)	1	0	19
	Clobetasol	10 (53%)	7 (37%)	2	0	19
Not stated	Diffucortolone	1	4	0	0	5
	Clobetasol	0	5	0	0	5

therefore undertaken. The results are shown in Table 4. Although the numbers in these subgroups are small, no statistically significant differences could be found between the two compounds for any of the diagnostic categories.

Systemic and local side-effects were recorded; 11 patients of the 354 (3.1%) participating in the study reported complaints. Eight of these were of secondary infection, described as light in 5 cases, heavy in 2, and 'superinfection' in 1. Six patients developed secondary infections on both sides; one patient (lightly infected) only on the clobetasol-treated side; and a further patient (heavily infected) only on the Dfv 0.3%-treated side. One case of mild burning on the clobetasol-treated side was reported, and one patient developed erysipelas of the leg on the side treated with Dfv 0.3%. Finally, one physician reported that atrophy of the skin had occurred in a 26-year-old man with psoriasis who had previously received topical steroid therapy; the atrophy occurred equally on both sides.

TABLE 4. Analysis of 111 patients with severe lesions by diagnosis

		Physician's assessment				
		Healed	Marked improvement	Slight improvement	Static	Total
Psoriasis	Diffucortolone	5 (14%)	21 (58%)	9 (25%)	1 (3%)	36
	Clobetasol	5 (17%)	23 (64%)	6 (17%)	1 (3%)	36
Atopic dermatitis	Diffucortolone	6 (38%)	8 (50%)	2 (13%)	0	16
	Clobetasol	6 (38%)	6 (38%)	4 (25%)	0	16
Eczema	Diffucortolone	18 (36%)	22 (44%)	9 (18%)	1 (2%)	50
	Clobetasol	17 (34%)	25 (50%)	7 (14%)	1 (2%)	50
Other	Diffucortolone	0	9	0	0	9
	Clobetasol	0	9	0	0	9

DISCUSSION

This study showed that in the treatment of a wide range of chronic dermatoses a new topical corticosteroid, Dfv 0.3%, was as effective as clobetasol, the most commonly prescribed potent corticoid, which is used especially for severe lesions. Although it was appreciated that higher cure rates could

be obtained with either compound by a longer period of treatment, it was considered that 3 weeks was sufficient to obtain a meaningful distinction between the compounds, if any such distinction existed, and that with a total dose of up to 100 g of each ointment (200 g total for each patient over the 3 weeks' therapy) further prolongation of treatment might cause severe depression of adrenal function. Thus both the length of treatment and the total dose were circumscribed by considerations other than that of achieving the maximal possible cure rate, and were in any case greater than that which is usual in clinical practice.

Since both compounds are recommended for severe lesions, particular attention was focused on the analysis of these lesions, both overall and by diagnostic category, in order to determine whether any real differences existed between them, but none was observed. These results indicate that for either eczema or psoriasis, and whatever the grade of severity of the lesion, Dfv 0.3% and clobetasol have a similar level of clinical efficacy. This work supports the investigative technique of Löffler (1978) which also indicated that, in the psoriasis plaque test, these two compounds were of similar efficacy. Moreover, the large numbers of patients investigated in this study gives confidence that no significant differences between the two compounds are present, since differences in efficacy as small as 6% are observable at statistically significant levels.

Furthermore, the incidence and severity of side-effects were remarkably similar for the two compounds with only 11 patients (3.1%) with complaints, of which 8 were readily treatable secondary infections. Seven patients had local side-effects equally on the two sides and of the remaining 4 patients, 2 had complaints on the clobetasol-treated side and 2 on the Dfv 0.3%-treated side.

Thus the choice of compound to be prescribed should be determined either by an assessment of previous clinical response—including the occurrence of side-effects—to one of the compounds, or by the amount of systemic effect in terms of adrenal suppression which might be produced. The work of Keczkas *et al.* (1978) indicated that far greater suppression of adrenal function occurred with the use of clobetasol than with Dfv 0.3%,—possibly due to the minimal amount of absorption of Dfv 0.3% from either intact or damaged skin (Täuber, 1976)—and our work on the systemic effects of these compounds (to be reported later) confirms these findings.

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REFERENCES

- KAPP, J.-H., KOHN, H., CASALS-STENZEL, J., TÖPERT, M. & GERHARDS, E. (1976) Studies on the pharmacology of 6 α , 9-difluor-11 β -hydroxy-16-methyl-21-valeryloxy-1,4-prenadien-3,20-dion (Diflucortolonvalerianat) *Arzneimittel-Forschung*, **26**, 1463.
- KECZKAS, K., TEASDALE, P., WISEMAN, R.A. & MUGGLESTONE, C.J. (1978) Plasma cortisol values after topical application of diflucortolone valerate (0.3%) or clobetasol propionate (0.05%) in psoriatic patients. *British Journal of Dermatology*, **99**, 415.
- LÖFFLER, O. (1978) Vergleichende Untersuchungen zur Wirksamkeit von Diflucortolon-valerianat 0.3% ig als W/Ö-Emulsion mit verschiedenen Handelspreparaten im Psoriasis-Plaque-Test. *Arzneimittel-Forschung*, in press.

Plasma cortisol values after topical application of diflucortolone valerate (0.3%) or clobetasol propionate (0.05%) in psoriatic patients

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SUMMARY

A double-blind randomized study to compare the plasma cortisol values at both 9.00 a.m. and 12 midnight following topical application of 10 g daily for 7 days of either diflucortolone valerate 0.3% (Nerisone Forte) ointment or clobetasol propionate 0.05% (Dermovate) ointment in 20 hospital in-patients suffering from severe psoriasis, showed that clinically both compounds behaved as potent, highly active topical preparations and caused rapid clinical improvement.

Diflucortolone valerate 0.3% caused only slight and non-significant depression of mean plasma cortisols. On the other hand, clobetasol caused an immediate, persistent and statistically significant depression of the 9.00 a.m. cortisol values, which appeared to recover towards normality only on the third day after therapy had been withdrawn. The difference between these 2 compounds was found to be statistically significant ($P < 0.05$).

From these observations, it is concluded that diflucortolone valerate 0.3% ointment suppresses adreno-cortical function to a significantly lesser extent than clobetasol propionate 0.05% ointment.

Diflucortolone valerate 0.3% is a potent topical corticosteroid which has been shown in a number of investigations, usually in predominantly severe skin conditions, to be comparable in efficacy to clobetasol propionate 0.05% (Löffler, 1978; Wendt, Mugglestone & Wiseman, 1978). Since the amount of adrenal suppression which may occur with clobetasol propionate, especially if used in excess or for long periods, has been the subject of some concern (Carruthers, August & Staughton, 1975; Feiweil & Kelly, 1974), it seemed of importance to investigate the amount of adrenal suppression which may be produced by diflucortolone valerate 0.3%. It was considered that a comparative study in two groups of patients, with similar lesions, treated with equal amounts of the two preparations and evaluated by measuring plasma cortisol levels, would give useful information on the comparative safety of diflucortolone valerate 0.3%.

PATIENTS AND METHODS

Twenty hospitalized patients suffering from severe psoriasis were investigated by having estimations of plasma cortisol before, during and after application of either diflucortolone valerate 0.3% ointment or clobetasol propionate 0.05% ointment, patients being randomly allocated to one of the two treatment regimes. Patients were those with chronic stable lesions covering 20–40% of body surface, and informed consent for the study was obtained.

Patients were excluded from the study if any of the usual contraindications to local steroid therapy were present, and also if they were receiving oral steroids, had received topical steroid therapy during the past month, were under 18 years of age, were pregnant, or were suffering from wet and infected lesions. No systemic therapy was allowed during the period of investigation. The two ointments were randomly allocated to patients and supplied in identical tubes labelled only with the patient's trial numbers. Each ointment was applied twice daily, and since many of the patients had widespread lesions it was decided, on grounds of safety, to limit the amount of steroid ointment used to 10 g daily. Any lesions requiring treatment in excess of this were treated with non-steroid ointments. Both the degree of clinical improvement and side-effects were recorded.

Plasma cortisol levels were taken at standard times daily throughout the trial period—at 9.00 a.m. and 12.00 midnight—and were measured by a fluorimetric method (James, Mattingly & Daly, 1971).

Base-line cortisol levels were provided by an initial 2-day pre-treatment period, together with the 9.00 a.m. cortisol level on the third study day. Immediately following this third estimation, topical therapy was commenced, and continued for a total of 7 days of treatment, in order to observe if any depression of plasma cortisol levels occurred. This was followed by a 3-day post-treatment period to determine, if depression had occurred, whether or not there was any delay in return to normal.

RESULTS

The overall clinical impression was that both steroids were potent, highly active, topical preparations, causing rapid improvement, and in some cases total healing of the psoriatic lesions.

No adverse reactions were reported.

Examination of individual patients' plasma cortisol levels from the treatment period as a whole compared to the pre-treatment period showed that in the clobetasol propionate group a statistically significant reduction occurred in 2 patients out of 10 from the 9.00 a.m. estimations, and 3 patients

TABLE 1. Mean plasma cortisol values and standard deviation for the patients treated with diflucortolone valerate 0.3% ointment

Regime:	Pre-treatment			Treatment period						Post treatment		
Day:	1	2	3	4	5	6	7	8	9	10	11	12
<i>9.00 a.m. cortisol values</i>												
Mean	348	320	273	278	277	301	274	304	325	373	342	319
s.d.	93	137	73	88	85	53	84	143	188	260	100	140
<i>12 midnight cortisol values</i>												
Mean	154	147	156	133	114	134	130	147	166	177	177	178
s.d.	54	58	72	64	49	51	55	56	77	109	91	103

out of 10 from the midnight estimations; some of these reductions persisted into the post-treatment period. From the diflucortolone valerate 0.3% group, no statistically significant reduction occurred in any individual patient.

Examination of mean plasma cortisol levels, together with standard deviations, from the group of patients treated with diflucortolone valerate 0.3% ointment, which are presented in Table 1, showed no statistically significant reduction from either 9.00 a.m. or 12 midnight values in any treatment day compared to the pre-treatment mean. Similar examination from the clobetasol propionate group, which is presented in Table 2, showed a statistically significant reduction for every treatment day as well as the first post-treatment day from the 9.00 a.m. estimations, and from 3 treatment days from the midnight values, as compared to pre-treatment means. This analysis was performed by means of an analysis of variance.

TABLE 2. Mean plasma cortisol values and standard deviation for the patients treated with clobetasol dipropionate

Regime:	Pre-treatment			Treatment period						Post treatment		
Day:	1	2	3	4	5	6	7	8	9	10	11	12
<i>9.0 a.m. cortisol values</i>												
Mean	413	473	412	312†	297‡	312†	334*	339*	324†	339*	376	383
s.d.	133	147	176	129	121	107	122	116	110	127	122	143
<i>12 midnight cortisol values</i>												
Mean	236	229	174	159	148*	136*	154	156	149*	159	165	226
s.d.	177	117	82	107	93	84	106	103	121	110	111	134

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

The comparative changes in group mean plasma cortisol levels from the 9.00 a.m. estimations have been calculated as a percentage of the respective pre-treatment values, and these are shown in Table 3, together with the asterisks to indicate the probability values. The 12.00 midnight estimations have been expressed in the same way in Table 4.

TABLE 3. Comparative changes in mean plasma cortisol levels, during the treatment and post-treatment phases, expressed as a percentage of the mean pre-treatment value

Treatment used and timing of cortisol	Pre- treatment	Treatment period						Post-treatment		
	1-3	4	5	6	7	8	9	10	11	12
Difluocortolone 9.00 a.m.	100	88.2	89.1	95.5	89.5	97.8	104.2	113.1	108.3	101.9
Clobetasol 9.00 a.m.	100	72.1†	68.6‡	72.1*	77.1*	78.3*	74.6†	78.3*	86.8	88.5

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

TABLE 4. Comparative changes in mean plasma cortisol levels, during the treatment and post-treatment phases, expressed as a percentage of the mean pre-treatment value

Treatment used and timing of cortisol	Pre- treatment	Treatment period							Post-treatment		
	1-2	3	4	5	6	7	8	9	10	11	12
Difflocortolone 12.00 midnight	100	104.0	88.5	75.7	89.3	86.7	98.7	110.7	117.8	117.7	118.5
Clobetasol 12.00 midnight	100	74.9	68.4	63.7*	58.5*	66.3	67.1	64.1*	68.4	71.0	97.2

* $P < 0.05$.

DISCUSSION

In the group treated with diflucortolone valerate 0.3%, neither any of the individual patients' treatment period, nor the mean of the whole treatment period for all the patients grouped together, showed any statistically significant depression when compared to pre-treatment levels. Although a drop in plasma cortisol levels was seen with this compound, with the numbers studied it did not reach levels of statistical significance.

On the other hand, three patients out of ten treated with clobetasol propionate showed highly significant depression during treatment and also post-treatment periods. Furthermore, the group mean of 9.00 a.m. plasma cortisol levels from every individual treatment day, as well as the first post-treatment day, in patients treated with clobetasol propionate, showed a statistically significant depression as compared to mean pre-treatment levels. Similarly in this group, the midnight plasma cortisol levels were statistically significantly reduced during treatment on days 5, 6 and 9.

Thus, while lack of a significant reduction in plasma cortisol levels with diflucortolone valerate 0.3% may be partly attributable to the size of the study, it was calculated that the reduction was significantly smaller ($P < 0.05$) than that seen with clobetasol propionate despite the similarity of lesions treated and the fact that the patients received equal amounts of the two ointments. It is therefore concluded that on grounds of safety, diflucortolone valerate 0.3% is the topical corticosteroid of choice, when a potent preparation is required.

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REFERENCES

- CARRUTHERS, J.A., AUGUST, P.J. & STAUGHTON, R.C.D. (1975) Observations on the systemic effect of topical clobetasol propionate (Dermovate). *British Medical Journal*, **iii**, 203.
- FEIWEL, M. & KELLY, W.F. (1974) Adrenal unresponsiveness associated with clobetasol propionate. *Lancet*, **ii**, 112.
- JAMES, V.H.T., MATTINGLY, D. & DALY, J.R. (1971) Recommended method for the determination of plasma corticosteroids. *British Medical Journal*, **ii**, 310.
- LÖFFERER, O. (1978) Vergleichende Untersuchungen zur Wirksamkeit von Diflucortolon-valerianat 0.3% ig als W/Ö-Emulsion mit verschiedenen Handels-präparaten im Psoriasis-Plaue-Test. *Arzneimittel Forschung*, in press.
- WENDT, H., MUGGLESTONE C.J. & WISEMAN, R.A. (1978) A study of the comparative efficacy of Diflucortolone valerate 0.3% ointment and clobetasol propionate 0.05% ointment. *British Journal of Dermatology*, **99**, 409.

Failure of metronidazole treatment in Ethiopian mucocutaneous leishmaniasis

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SUMMARY

Metronidazole was found to be of no value in the treatment of five cases of Ethiopian mucocutaneous leishmaniasis.

Treatment of mucocutaneous leishmaniasis in Ethiopia is still unsatisfactory. The drug of choice, pentamidine isothionate, is toxic and requires in-patient care (Bryceson, 1968, 1970, 1976). Moreover, relapse may occur after initial improvement (Naafs *et al.*, 1978).

Metronidazole, a relatively non-toxic drug, has been used with success in the treatment of Mexican cutaneous leishmaniasis (Beltran *et al.*, 1967; Long, 1973). Similar success was claimed from Afghanistan (Pedersen & Sawicki, 1975).

This study examines the effect of metronidazole treatment in patients with Ethiopian mucocutaneous leishmaniasis.

MATERIAL AND METHODS

Five patients (2 females, 3 males) with Ethiopian mucocutaneous leishmaniasis were selected from the outpatient clinic of the Addis Ababa Leprosy Hospital. They were clinically free from any other overt disease. The clinical diagnosis of leishmaniasis was confirmed by the detection of the *Leishmania* parasites histologically from the lesion. In most cases of Ethiopian mucocutaneous leishmaniasis it is not possible to isolate parasites from the lesion (although they are there) by culturing in the conventional NNN medium. The treatment consisted of metronidazole (Mezil, Epharm, Ethiopia) in a dose of 500 mg three times daily. The treatment continued for 4–8 weeks. Clinical changes were recorded and biopsy results were compared before and after treatment. The histological examinations were done on Haematoxylin and Eosin stained sections.

RESULTS

Metronidazole did not cause any change in the lesions of the five treated patients as assessed clinically and histologically. Two of the patients complained of burning sensations in the lesions during the

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treatment period, but no objective changes could be detected. No serious side effects were recorded.

After the treatment period with metronidazole all patients received the routine treatment with pentamidine isothionate (May and Baker Ltd) on which they went into remission.

DISCUSSION

The present study strongly suggests that metronidazole is of little value in the treatment of Ethiopian mucocutaneous leishmaniasis. This is in agreement with the observation of Kerdal Vegas (1975) who reported that metronidazole was only effective in Mexican cutaneous leishmaniasis, but not in mucocutaneous cases.

A trial treatment with metronidazole in two cases of Ethiopian diffuse cutaneous leishmaniasis showed inconclusive results. Clinically there was initial improvement, which in one case could be confirmed histologically, but no complete remission occurred (Belehu, unpublished observation). One explanation for this finding is that perhaps mucocutaneous leishmaniasis and diffuse cutaneous leishmaniasis are caused by different strains of *Leishmania*. However, the available evidence to date suggests that the different forms of cutaneous leishmaniasis are due to differences in the host response to the parasite (Bray & Bryceson, 1969; Convit, 1974).

The possibility that different strains of *Leishmania aetiopica* indeed cause different forms of Ethiopian cutaneous leishmaniasis is currently been re-examined in our laboratory with more sensitive techniques.

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REFERENCES

- BELTRAN, F.H., GUTTIERREZ, M. & GIAGI, F.F. (1967) Utilite du metronidazole dans le traitement de la Leishmaniose cutane Mexicaine. *Bulletin de la Société de Pathologie exotique*, **50**, 61.
- BRAY, R.S. & BRYCESON, A.D.M. (1969) Studies on the immunology and serology of leishmaniasis. VIII. The identity of strains of *Leishmania* from Ethiopian diffuse cutaneous leishmania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **63**, 524.
- BRYCESON, A.D.M. (1968) Pentamidine induced diabetes mellitus. *East African Medical Journal*, **45**, 110.
- BRYCESON, A.D.M. (1970) Diffuse cutaneous leishmaniasis in Ethiopia. II. Treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **64**, 369.
- BRYCESON, A.D.M. (1976) Tropical dermatology: cutaneous leishmaniasis. *British Journal of Dermatology*, **94**, 227.
- CONVIT, J. (1974) Leprosy and leishmaniasis: similar clinical-, immunological-, pathological models (Kellesberger memorial lecture). *Ethiopian Medical Journal*, **12**, 187.
- KERDAL-VEGAS, F. (1975) American leishmaniasis. In: *Clinical Tropical Dermatology* (Ed. by O. Canizares), p. 197. Blackwell Scientific Publications, Oxford.
- LONG, P.I. (1973) Cutaneous leishmaniasis treated with metronidazole. *Journal of the American Medical Association*, **223**, 1378.
- NAAFS, B., BELEHU, A., WARNDORFF, J. & WHEATE, H.W. (1978) Treatment of cutaneous leishmaniasis in the Addis Ababa Leprosy Hospital (in preparation).
- PEDERSEN, J.K. & SAWICKI, S. (1975) Metronidazole therapy for cutaneous leishmaniasis. *Archives of Dermatology*, **111**, 1343.

Case Reports

An hereditary syndrome of lumpy scalp, odd ears and rudimentary nipples

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SUMMARY

A syndrome, consisting of a congenital abnormality of the scalp, ears and nipples inherited as an autosomal dominant trait, is reported. Ten affected members of a family over five generations are recorded and the four living affected members are described in detail. A review of congenital localized aplasias of skin suggests that this may be a new syndrome.

The propositus—a 51-year-old lady (Case 31)—was referred to the Dermatology Department in July 1976, because of raised nodules over the back of the scalp, that had been present for many years. She was also noticed to have curiously shaped ears. The patient mentioned that her father had similarly shaped ears and scalp lesions. It became apparent that this distinctive combined abnormality was well known in this family and the family tree (Fig. 1) was traced. The following members of the family were interviewed: 13, 14, 18, 21, 31, 33 and 35, of these 14, 21, 31 and 35 were affected. Information, including photographs of a deceased member of the family, was obtained and the medical records of two affected deceased members (Cases 15, 56) were reviewed.

The family

The kinship consists of 56 direct descendants of one affected woman (Case 1) who came from Somerset, England, and who died in 1918. No further information could be obtained about her parents or siblings. She was known, however, to have distinctively shaped ears and lumps on the back of her scalp.

Of her seven children only one was affected (Case 2) who was said to have died in 1938 of cancer of the throat. Case 2 was the mother of three affected sons and one affected daughter (Cases 10, 14, 15 and 17), but only one of her affected children (Case 14) survives. Of Case 2's 22 grandchildren, only those descended through affected persons have themselves been affected and so the trait which has been transmitted in an autosomal dominant fashion may not be transmitted to further generations

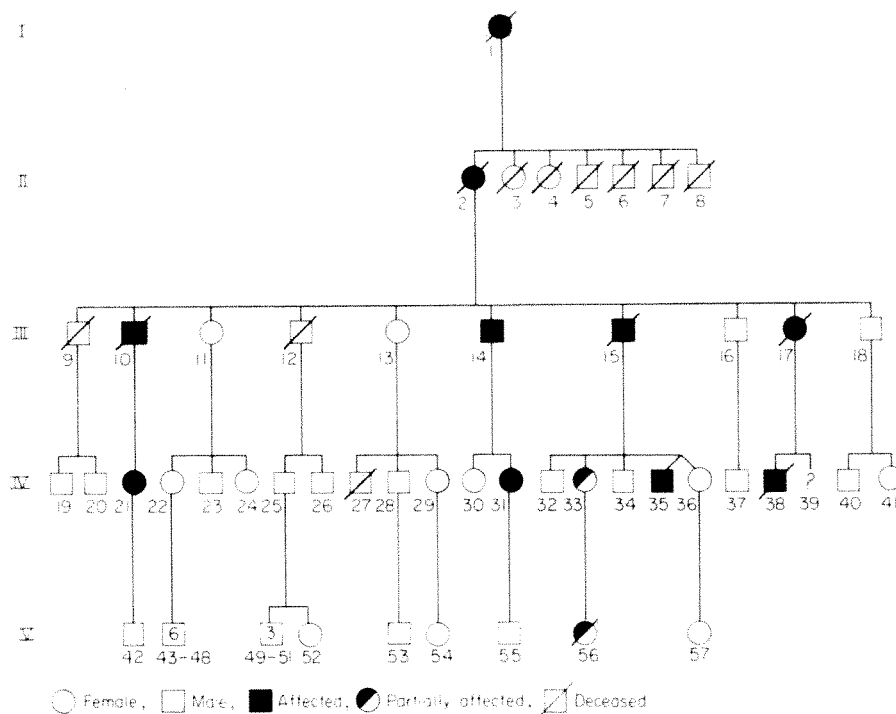


FIGURE 1. The family tree.

CASE REPORTS

Case 31

This 51-year-old lady has been aware of malformed ears and scalp lesions since childhood. She has always had scanty pubic hair and absent axillary hair. In 1948 diabetes mellitus was diagnosed and she has been controlled with insulin (Actrapid 32 units mane and 24 units nocte). She developed a pulmonary embolism in 1949, and in 1951 was investigated because of obesity, hair loss and loss of libido. Rudimentary nipples were noted. Her breasts had not enlarged during pregnancy and lactation had not occurred. No other endocrine abnormality has been discovered. She has been treated for hypertension since 1970.

When seen in 1976 she had several raised firm nodules over the posterior aspect of the scalp and her ears showed the abnormal configuration described below. In addition rudimentary nipples and scanty secondary sexual hair, brittle thumb nails and slight bilateral webbing of the second and third toes were noted.

The scalp nodules were symptom free until January 1977 when the patient became aware of a nodule which clinically showed central necrosis. The histology of an uncrusted nodule is described below.

Her sister (Case 30) also has diabetes, is infertile and has an 'undeveloped uterus'. Her breasts and nipples are, however, normal.

Case 14

This man aged 76 (father of Case 31 above) is the only living affected member of generation three.

He has raised firm uneven nodules on the posterior part of his scalp which have been present since birth. He has been a diabetic since 1957 controlled at present with 32 units of lente insulin and he also has hypertension controlled with methyl dopa 250 mg twice daily. On examination his pinnae show a similar abnormality to that of his daughter; he has rudimentary nipples and scalp nodules. A scalp nodule has been biopsied and is described below.

Case 35

This man aged 50 is the affected son of Case 15. His elder sister (Case 33) reports that following his birth the affected area of the scalp 'was like raw beef' and this area was kept protected. Later during infancy the affected area healed over. Since then two small raised areas on the back of his head have always been bald, with a small bridge of normal hair between. His general health has always been good. He has a non-identical twin sister who is unaffected. His scalp nodules and ear configuration are similar to those of Cases 31 and 14. He has no children.

His father, Case 15, died in 1939 aged 47 of septicaemia following an infection of a cut finger. The post mortem report does not comment on his scalp or ears but he was known to have had the same changes of bald lumps on the back of his head and the same unusual shape of ears. The sister of Case 35 (Case 33) has ears of a similar configuration though no other abnormalities. Her daughter, Case 56, died aged 25 of hypertensive heart failure secondary to glomerulonephritis. Although Case 56 had no ear abnormality there was a single typical hairless lump on the back of her scalp.

Case 21

This lady aged 51 is the affected daughter of Case 10. She has two small bald scarred flat areas on the posterior aspect of the scalp which have been present since birth. There is only a minor change in the helix of the pinnae. Although she has nipples she was unable to breast feed.

Her only son, Case 42, is unaffected. Case 10 (the affected father of Case 21) died in 1975 aged 72 of leukaemia. His daughter reports that he had two separate bald patches associated with lumps in the skin over the back of the head. A photograph shows that there was a change in his pinnae with some folding over of the helix. He was said to have had 'absent nipples'.

Further family information

Case 17 was affected with both scalp and ear changes. She had an affected son, Case 38, and died in childbirth at her second pregnancy. The child, Case 39, survived, was adopted and apparently taken to South Africa. There is no information as to whether this child was affected and this poses the intriguing question of whether the trait persists elsewhere. The affected first son, Case 38, died aged 22 while serving in the Army, apparently of a brain haemorrhage.

The scalp abnormality (Figs 2 and 3)

In and adjacent to the midline, over the posterior aspect of the scalp, there are raised firm nodules, which are not covered by hair. These areas are raw at birth and heal over in early childhood.

These lesions have been seen in Cases 14, 31 and 35. In Case 21 there are two bald scarred flat and round areas at the same site. Skull X-rays of our patients were normal.

The scalp lesions of Cases 14 and 31 were biopsied. The dermis of both scalp lesions shows what appears to be an excess of normal connective tissue. The arrangement and characteristics of the connective tissue bundles does not suggest a morpheo-like change, hypertrophic scar or keloid. In some areas there are parallel bands of fibres which interlace irregularly suggestive of scarring but this feature does not predominate and overall there are no firm diagnostic features. There appears to be some replacement of fat by the connective tissue. There is also a striking absence of pilosebaceous



FIGURE 2. The scalp abnormality: close-up (Case 31) before biopsy.

apparatus and sweat coils. Special stains and orcein staining demonstrate a normal distribution of elastic fibres in the connective tissue mass. There is elastotic degeneration in the papillary and upper dermis of Case 14. Direct immunofluorescence has shown a faint linear band of IgM at the basal layer; the significance of this is unknown.

DISCUSSION

The abnormality in the family described here bears some similarity to 'congenital localized aplasia of the skin' (CLAS) (Bart *et al.*, 1966). The most frequently described lesion in CLAS is of a coin shaped solitary area at the vertex near the midline devoid of hair and skin (Bart, 1973). This crusts over shortly after birth forming a fine depressed hairless scar, similar to the scalp lesions in Case 21, though the nodular lesions in our other patients are much larger and raised. The histological picture



FIGURE 3. The scalp abnormality: general view (Case 31) showing biopsied area.

of our patients' lesions was not that of keloid scar tissue, which has been described in areas of congenital defects of the scalp (Moschella, 1962). However, the history of the patients being born with a 'raw scalp' area which later heals over suggests that the scalp lesion may be a variant of CLAS.

In previous studies of congenital familial scalp defects (Johnsonbaugh, Light & Sutherland, 1965) abnormalities of underlying bone have been demonstrated at birth but serial X-rays have shown spontaneous healing of the bone defect by 6 months (Hodgman, Mathies & Levan, 1965). Therefore although the skull X-rays of our patients were normal, this does not preclude the possibility that at birth there may have been radiological changes.

External ears (Figs 4 and 5)

The pinnae of our patients have the following abnormal characteristics; the tragus, antitragus and lobule are small or in the most severe form, rudimentary. The superior edge of the helix is 'turned over' to an exceptional degree; for example, the helix of the right ear of Case 31 is turned over 1.5 cm. The antihelix ridge is flattened. These changes are severe in Cases 31 and 14, less obvious in Case 35, and minimal in Case 21.



FIGURE 4. The abnormal external ear; lateral aspect of left ear (Case 14).

Nipples (Fig. 6)

The nipples are rudimentary or absent, with areolae only present. In the most severe form, Case 31, there is a flat brown round area 1 cm in diameter at the normal nipple site. This nipple abnormality is also seen in Case 14, and was apparently present in Case 10.

Diabetes mellitus

Three members of the family have diabetes, the family group Cases 14, 30 and 31. Case 12 was also apparently a diabetic, but he had no other features described above.

Genetic aspects

We have not found any previous record of this curious association between scalp lesions and mal-developed ears. The mode of inheritance appears to be autosomal dominant with incomplete penetrance and there is an apparent association with incomplete nipple formation. Although four members of the family have had diabetes there seems to be no clear correlation between this and the inheritance of the other features.

In no previous reports of CLAS occurring in families are the ears or nipples involved (Bart *et al.*, 1966; Johnsonbaugh *et al.*, 1965; Hodgman, 1965). Frank & Ruby (1957) described a mother of two children with a congenital defect of the scalp, but there were no associated abnormalities, and their review only contains two previous reports of members of the same family affected. In these

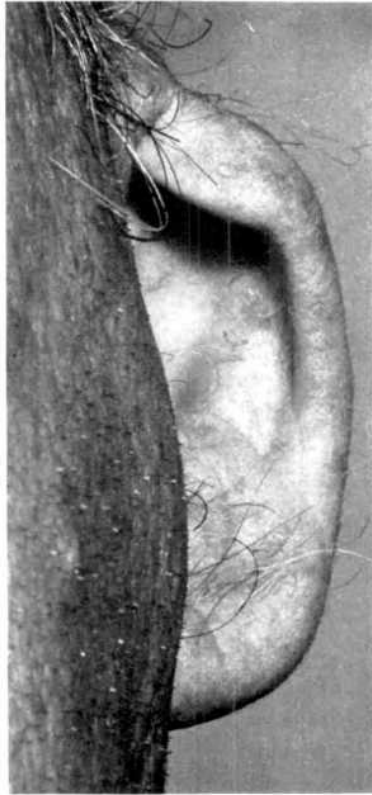


FIGURE 5. The abnormal external ear; anterior aspect, left ear (Case 14).



FIGURE 6. A rudimentary nipple (Case 31).

families and in a family described by Freud, Rhodes & Weisz (1945) only cases in a maximum of two generations are described, though Tisserand-Perrier (1953) has described scalp defects occurring in three generations of one family.

There is no mention of a similar syndrome in Gorlin & Pindborg's (1976) book of 'Syndromes of the head and neck' or in Cockayne's (1953) or McKusick's (1975) publications on genodermatoses. Smith (1976) gives a photograph of an incompletely outfolded scaphahelix which he considers should be classed as a minor variant and not as an anomaly. He also gives a photograph of punched out lesions in the scalp of a neonate, presumably similar to that quoted by Bart (1973). No association between the two is recorded.

Aetiology

The aetiology of congenital defects of the scalp has been discussed at length but these reviews do not discuss the associated abnormalities seen in the family described (Frank & Ruby, 1957; Freud *et al.*, 1945; Pers, 1963; Savage, 1956).

It has been suggested that there may be an association with amniotic bands and that the formation of these amniotic bands may be genetically induced (Pers, 1963). This mode of formation, however, seems unlikely in our patients where the defects are widely spaced and it seems unlikely that there is any mechanical damage *in utero*. There may, however, be a localized arrest of development *in utero* (Savage, 1956). It seems more likely that there are related genes affecting development of all sites affected in the kindred described.

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REFERENCES

- BART, B.J., GORLIN, R.J., ANDERSON, V.E. & LYNCH, F.W. (1966) Congenital localised absence of skin and associated abnormalities resembling epidermolysis bullosa. *Archives of Dermatology*, **93**, 296.
- BART, B.J. (1973) Localized absence of skin. In: *Birth Defects* (Ed. by Daniel Bergsman), p. 582. Williams and Wilkins Co., Baltimore.
- COCKAYNE, E.A. (1933) *Inherited Abnormalities of the Skin and its Appendages*. Oxford University Press.
- FRANK, L. & RUBY, A. (1957) Familial congenital defect of the scalp. *Archives of Dermatology*, **75**, 266.
- FREUD, P., RHODES, A.W. & WEISZ, A. (1945) Hereditary skin defect in the newborn infant. *Journal of Paediatrics*, **27**, 591.
- GORLIN, R.J. & PINDBORG, J.J. (1976) *Syndromes of the Head and Neck*, 2nd edn. McGraw Hill, New York.
- HODGMAN, J.E., MATHIES, A.W. & LEVAN, N.E. (1965) Congenital scalp defects in twin sisters. *American Journal of Diseases of Children*, **110**, 293.
- JOHNSONBAUGH, R.E., LIGHT, I.J. & SUTHERLAND, J.M. (1965) Congenital scalp defects in father and son. *American Journal of Diseases of Children*, **110**, 297.
- MOSCHELLA, S.L. (1962) Congenital defects of scalp with keloid formation. *Archives of Dermatology*, **86**, 63.
- McKUSICK, V.A. (1975) *Mendelian inheritance in Man*. The John Hopkins University Press.
- PERS, M. (1963) Congenital absence of skin: pathogenesis and relation to ring-constriction. *Acta Chirurgica Scandinavica*, **126**, 388.
- SAVAGE, D. (1965) Localized congenital defects of the scalp. *Journal of Obstetrics and Gynaecology of the British Empire*, **63**, 351.
- SMITH, D.W. (1976) *Recognisable Patterns of Human Malformation*, pp. 448-9. W.B. Saunders Company, London.
- TISSERAND-PERRIER, M. (1953) Transmission pendant plusieurs generations d'une aplasie cutt anée circonscrite du vertex. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **60**, 77.

Follicular atrophoderma with multiple basal cell carcinomas (Bazex)

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SUMMARY

Five patients from a single family are reported who have an inherited condition of which the main features are follicular atrophoderma, abnormalities of scalp hair and multiple basal cell carcinomas. These abnormalities are consistent with the syndrome described by Bazex *et al.* (1964). The pattern of inheritance of this condition is discussed.

Follicular atrophoderma associated with basal cell carcinoma, hypotrichosis and localized or generalized hypohidrosis was first described by Bazex, Dupre & Christol (1964). Further cases have recently been reported (Viksnins & Berlin, 1977). We have documented what we believe are the first reported British cases of this rare inherited syndrome. In addition, we describe structural changes in the hair of our patients which have not been recorded previously.

CASE REPORTS

Case 1

A 28-year-old man presented with multiple skin tags on the neck. The skin of the face, elbows and backs of hands was noted to be prominently pitted (Figs 1 and 2). The normally slightly funnel shaped opening of the pilosebaceous unit was greatly exaggerated and the associated hairs were hypotrophic or absent. There were no pits visible on the palms or soles. Scalp hair was golden brown and sparse. There was a male pattern alopecia. Many of the hairs had a spangled appearance reminiscent of pili torti. Close examination of his skin revealed no basal cell carcinomas or other skin tumours.

Our patient informed us that he had never produced sweat on the face and neck, even after the most severe exertion. He remarked that the skin pitting has been present since birth and was not considered unusual in his family. Indeed several of his relatives had undergone surgery for 'skin cancers'.

X-rays of skull, jaw and chest were normal. Routine haematological and biochemical screening was unremarkable. The serum calcium was within normal limits. The patient refused a skin biopsy but permitted the removal of scalp hairs for study. Whilst normal scalp hairs were present a proportion showed a striking twisting and flattening of the hair shaft. These appearances were well demonstrated by scanning electron microscopy (Fig. 3a and b).

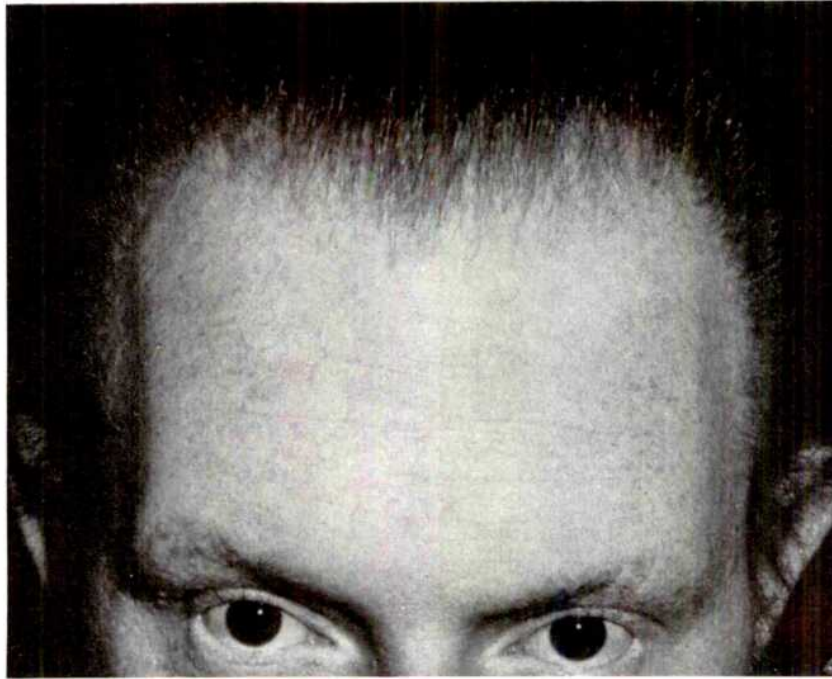


FIGURE 1. Prominent pitting of skin on the face.

Case 2

A 50-year-old woman, mother of Case 1. This patient exhibited precisely the same clinical features as her son. The abnormalities had been present since birth. In addition, since the age of 30 years nine basal cell carcinomas had developed on her face and required treatment with radiotherapy or surgery. On presentation a further superficial basal cell carcinoma was present on her right cheek.

Radiological and biochemical screening was unremarkable. Scanning electron microscopy of scalp hair showed features identical with Case 1. The basal cell carcinoma was excised and the diagnosis confirmed histologically. The lesion was in an area of follicular atrophoderma but no histological abnormality of the hair follicles was identified.

This patient provided detailed information from which the pedigree was drawn (Fig. 4). We have tabulated information concerning five affected members of the family.

DISCUSSION

The cases reported fit closely with the clinical pattern of the syndrome reported by Bazex *et al.* (1964). They differ in that the age of onset of the basal cell carcinomas was later (30 years) than that described by Viksnins & Berlin (1977) (15–26 years). Scalp hair from our patients showed marked twisting and flattening but this may not be unique since earlier investigators did not examine scalp hair with the scanning electron microscope.

The syndrome is quite distinct from that described by Gorlin & Goltz (1960). The follicular atrophoderma shown by affected patients is reminiscent of orange peel and quite unlike the characteristic pits which patients with Gorlin's syndrome have on the palms and soles. There are no associated



FIGURE 2. Prominent pitting of skin on the backs of hands.

TABLE 1. Summary of clinical features

Case no.	Age/sex	Basal cell carcinoma	Age at onset of BCC (years)	Follicular atrophoderma	Hypohidrosis	Hypotrichosis	Twisted and flattened hairs
1	28/M	None to date	—	Present	Face & neck	Present	Present
2	50/F	Many	30	Present	Face & neck	Present	Present
3	52/F	Many	29	Present	Face & neck	Present	Not examined
4	70/F	None	—	Present	None	None	Not examined
5	72/F	Many	30	Present	None	Present	Not examined

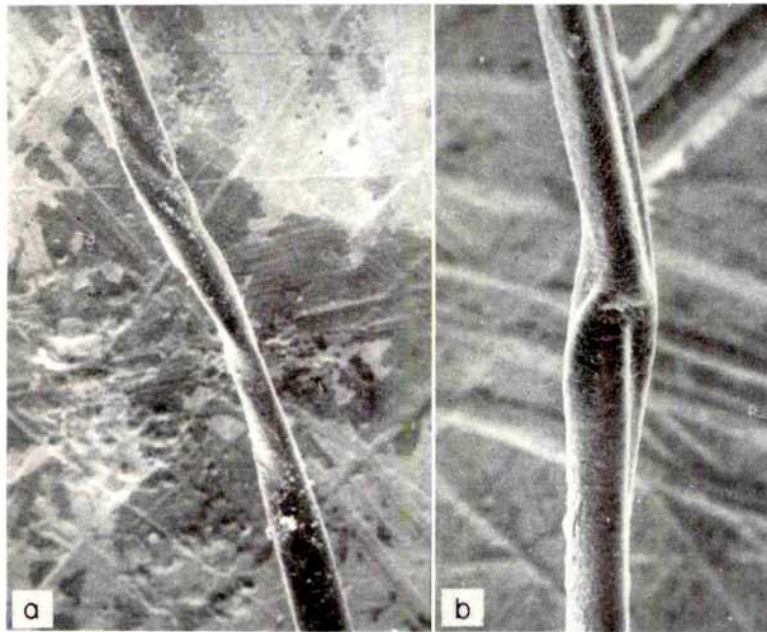
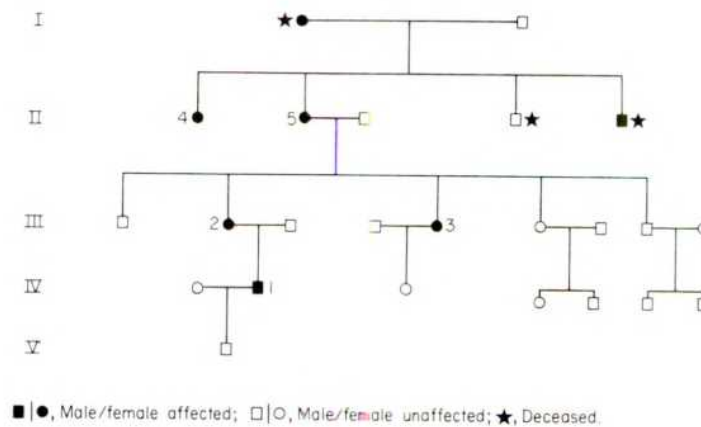


FIGURE 3. (a) Scanning electron micrograph of affected hair.
(b) Scanning electron micrograph of affected hair.



■/●, Male/female affected; □/○, Male/female unaffected; ★, Deceased.

FIGURE 4. Pedigree of affected family.

dental abnormalities. Viksnins & Berlin (1977) pointed out that follicular atrophoderma is a feature of the Conradi syndrome. However, none of the systemic and skeletal anomalies associated with the Conradi syndrome is found in patients like ours.

The pedigree is compatible with an autosomal or X-linked dominant pattern of inheritance. Male to male transmission has never been reported in any of the families studied and this makes an X-linked dominant pattern more likely. As can be seen from Table 1 the phenotypic expression of the cutaneous abnormalities is by no means constant. As far as the living members of our family are aware there is no history of consanguinity.

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We should like to thank Dr W.J.Cunliffe for his permission to report these patients and Mr J.Buckley of The University of Leeds Textile Department for performing the scanning electron microscopy.

REFERENCES

- BAZEX, A., DUPRÉ, A. & CHRISTOL, B. (1964) Génomodermatose complexe de type indéterminé associant une hypotrichose, un état atrophodermique généralisé et des dégénérescence cutanées multiples (épithéliomas basocellulaires). *Bulletin de la Société Française de Dermatologie et de Syphiligraphie*, **71**, 206.
- GORLIN, R.J. & GOLTZ, R.W. (1960) Multiple nevoid basal cell epitheliomas, jaw cysts and bifid rib: A syndrome. *New England Journal of Medicine*, **262**, 908.
- VIKSNINS, P. & BERLIN, A. (1977) Follicular atrophoderma and basal cell carcinomas. *Archives of Dermatology*, **113**, 948.

A new syndrome with pigmentation, scleroderma, gynaecomastia, Raynaud's phenomenon and peripheral neuropathy

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SUMMARY

A middle-aged man developed impotence, gynaecomastia, peripheral neuropathy, pigmentation, lymphadenopathy, scleroderma, malabsorption and Raynaud's phenomenon following prolonged exposure to trichlorethylene.

Some, but not all, of these features have previously been reported in vinyl chloride disease, and trichlorethylene is related chemically to vinyl chloride. Seven patients with a similar but not identical syndrome have been described in Japan and U.S.A., apparently unrelated to exposure to industrial toxins.

We here report a patient with a bizarre and puzzling combination of clinical features, which does not appear to have been previously described, although a somewhat similar but not identical syndrome has been recently reported in seven patients in Japan and U.S.A. (Meshkinpour, Myung & Kramer, 1977).

CASE REPORT

A Caucasian male maintenance engineer, born in 1930, presented in 1965 with gout of the great toe. He was treated with allopurinol and probenecid for several years, with symptomatic relief.

In March 1972 he developed numbness of the soles and burning sensation in the feet, and direct questioning revealed that he had been impotent for the past year. On examination, he had slight hepatomegaly, gynaecomastia, absence of both knee and ankle jerks, mild Rombergism and diminution of pain sensation below mid-shin level. The testes felt normal. A diagnosis of peripheral neuropathy was made. Investigation revealed a raised serum urate but no radiological evidence of gout. No cause was found for the neuropathy.

In January 1973 he was admitted for further tests because of a 2 stone loss of weight and axillary and inguinal lymphadenopathy was noted. A liver biopsy showed focal fatty changes with some histiocytic granulomata but special stains revealed no evidence of acid-fast bacilli, fungi or excessive iron

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deposition. A lymph node biopsy showed only reactive hyperplasia, and a second biopsy from a different site showed reactive hyperplasia with partial transformation to Castleman's angiofollicular hyperplasia (Dr J.Davies). This is a non-specific response in which the follicular germinal centres acquire leashes of abnormal laminated blood vessels (Harrison, 1976). Wade-Fite modified Ziehl-Neelson staining of hepatic and lymph-node specimens showed no evidence of *Mycobacterium leprae*. Plasma protein electrophoresis was normal, but serum immunoglobulins were slightly abnormal with IgA 109 mg/100 ml (N 140-400), IgG 590 mg/100 ml (N 800-1700) and IgM 210 mg/100 ml (N 50-200). Many other investigations were negative or normal. These included full blood count, plasma viscosity, urea and electrolytes, creatinine clearance, liver function tests, fasting blood sugar, serum iron and iron-binding capacity, auto-immune profile (including anti-nuclear factor), rheumatoid factor, W.R., skeletal survey, intravenous pyelogram, sternal marrow aspiration, iliac crest biopsy and bronchoscopy.

Because the neuropathy was slowly progressing, treatment was started in August 1973 with prednisone, 20 mg on alternate days. This treatment, which halted the deterioration in his neurological condition, has been continued to the present time, since stopping prednisone causes a relapse of the burning pain in the feet.

In January 1974 he was referred for dermatological opinion because of the gradual development of scleroderma. He gave a 2-year history of Raynaud's phenomenon of the hands following exposure to cold, and he complained of increasing muscle stiffness, and a sensation of generalized weakness. On examination he was found to have induration and a peculiar slate-blue discolouration of the skin especially of the hands and lower legs. There was mild bilateral pitting ankle oedema. There was no nailfold capillary dilatation, and the finger print pattern was not lost. A skin biopsy from the knee showed some hyperkeratosis and a diffuse round cell infiltrate in the dermis with some melanin in dermal macrophages, but the changes were non-specific. A second skin biopsy showed similar features and direct immunofluorescence was negative. A diagnosis of systemic sclerosis was considered, but tests for auto-immune profile, W.R., rheumatoid factor, cold agglutinins and cryoglobulins were negative, and he had normal chest and hand radiographs, barium meal and pulmonary function tests. Blood, urine and faeces were negative for porphyrins, and the urinary excretion of 5 HIAA was normal.

In 1975 he became increasingly tired and depressed with frequent headaches, and he also complained of the gradual onset of constipation with bulky, infrequent stools. Extensive investigation revealed malabsorption, with low serum iron, T.I.B.C., low red cell folate, low d-xylose excretion and abnormal Schilling test both with and without intrinsic factor. A duodenal aspirate revealed heavy bacterial growth (*Streptococcus faecalis* 1.2×10^8 organisms/ml). Multiple jejunal biopsies with a Quinton tube, 3 day faecal fatty acid excretion and ^{14}C -labelled glycocholate breath test were normal. Barium meal and follow through examination showed nonspecific features of malabsorption with a slow transit time to the caecum. Following treatment with metronidazole there was symptomatic improvement in the bowel habit, both Schilling tests as well as the duodenal aspirate became normal, and the d-xylose excretion showed improvement.

Further investigations in 1978 which have given normal results have included serum immunoglobulins, lumbar puncture, bone scan, skeletal survey, sternal aspirate, iliac crest trephine, and glucose tolerance test.

Work history

The patient has been intermittently exposed to carbon tetrachloride and trichlorethylene since 1947, when he joined the Royal Navy and trained as an aircraft mechanic. During his career in the Navy he travelled all over the world and consumed large daily quantities of alcohol (mainly rum). He left the Navy in 1970 and took a job in a helicopter factory where he was exposed to high concentrations

of trichlorethylene vapour throughout the working day. The patient denied any episodes of dizziness or sleepiness during exposure to the solvent however and he felt that many of his healthy colleagues were exposed to trichlorethylene fumes to a similar extent.

DISCUSSION

This patient presents a considerable diagnostic challenge. Over a 13 year period he has progressively developed gout, peripheral neuropathy, impotence, gynaecomastia, hepatic granulomata, reactive lymphadenopathy, scleroderma, pigmentation, Raynaud's phenomenon, muscle stiffness, depression and malabsorption due to abnormal intestinal flora. Many diseases produce varying combinations of several of these features, but we have been unable to find any diagnostic label to encompass them all. Some features of this case could represent an idiosyncratic reaction to trichlorethylene exposure. Vinyl chloride disease, which affects workers engaged in the production of polyvinyl chloride, produces a syndrome with Raynaud's phenomenon, scleroderma, involvement of liver, lungs and muscles and immune disturbance (Lange *et al.*, 1974). Other patients with this syndrome may complain of tiredness, limb pains and impotence (Walker, 1975, 1976). Vinyl chloride monomer is thought to be the aetiological agent in this disease, but many other low molecular weight, partially-polymerized materials are left in the reactor scrapings, and Dinman *et al.* (1971) suggested that the disease might be caused by several agents, each producing a specific component of the clinical picture. Our patient had never knowingly been exposed to vinyl chloride, but there is a close chemical similarity between vinyl chloride ($\text{CH}_2 = \text{CH Cl}$) and trichlorethylene ($\text{CHCl} = \text{CCl}_2$). Trichlorethylene is sometimes added to the reactors during polyvinyl chloride manufacture to produce the lower molecular weight polymers (Cook *et al.*, 1971). In vinyl chloride disease it has been suggested that the chemical combines with body proteins to render them antigenic (Milford Ward *et al.*, 1976). We have been unable to obtain definite evidence of immunological abnormality in our patient, although the generalized lymphadenopathy has been persistent.

Another chemical with a close similarity to trichlorethylene is perchlorethylene ($\text{CCl}_2 = \text{CCl}_2$). Lob (1957) described acrocyanosis following perchlorethylene poisoning and Sparrow (1977) has also described a patient with Raynaud's disease, muscle stiffness, scleroderma and impotence due to exposure to perchlorethylene.

Gastro-intestinal symptoms including anorexia and intolerance of fatty foods occur in trichlorethylene workers (Smith, 1966). Autonomic dysfunction has been suggested as a contributory cause (Grandjean *et al.*, 1955) and the small bowel bacterial overgrowth in our patient could be the result of impaired gut motility. Chronic exposure to trichlorethylene vapour is known to cause headache, fatigue, muscle weakness and pain, and impotence, but reviews of the toxicology of trichlorethylene do not mention the development of scleroderma or Raynaud's phenomenon (Smith, 1966; Defalque, 1961). Reinl (1957), however, reported the case of a young woman exposed to trichlorethylene for 2 years, who developed Raynaud's phenomenon, ankle oedema and widespread scleroderma. Trichlorethylene may also cause loss of memory, depression and mild psycho-organic syndromes (Grandjean *et al.*, 1955). Peripheral neuropathy due to trichlorethylene has been described (Stuber, 1931) but is now thought to have been due to impurities (Smith, 1966) and the relationship of our patient's neuropathy to trichlorethylene is uncertain.

The hepatic granulomata in our patient are unexplained, but the liver is no longer enlarged and liver function tests have been normal throughout. Allopurinol has been reported to cause hepatic granulomata which cleared following withdrawal of the drug (Simmons *et al.*, 1972) and our patient received allopurinol from 1965 until 1973, when his hepatomegaly was first discovered.

It is possible therefore that some of the clinical features in our patient could be due to prolonged

exposure to trichlorethylene vapour. Trichlorethylene is widely used in the engineering industry, however, and the paucity of previous case reports suggests that if the condition is due to trichlorethylene, it occurs only as a rare idiosyncratic reaction.

Another, perhaps more likely, possibility is that our patient is suffering from a variant of the bizarre syndrome recently described in seven patients in U.S.A. and Japan (Meshkinpour, Myung & Kramer, 1977). The major features in this multisystem disease of unknown origin were first described by Shimpo (1968). They include polyneuropathy, pigmentation, scleroderma, hirsutism, anasarca (with oedema of the extremities, ascites and pleural effusion), hepatosplenomegaly and lymphadenopathy, pseudotumour cerebri, gynaecomastia and impotence, plasma cell dyscrasia with monoclonal M-spikes, bone lesions with osteosclerotic changes, diabetes mellitus and polycythaemia. The condition occurs mainly in middle-aged subjects but exposure to industrial toxins such as trichlorethylene has not been reported, and three of the seven cases have been female. Although not every component of the syndrome has occurred in all of the reported cases, anasarca and diabetes mellitus have been considered hallmarks of the disease, and these features were absent from our patient. Our patient also had no evidence of splenomegaly, pseudotumour cerebri, osteosclerosis, polycythaemia or plasma cell dyscrasia, whereas he did have malabsorption due to bacterial overgrowth of the intestine. Further experience may enable us eventually to categorize our patient as a variant of Shimpo's syndrome, but we suggest that in similar cases careful attention should be paid to the possibility of exposure to industrial toxins.

REFERENCES

- BAUER, M. & RABENS, S.F. (1977) Trichlorethylene toxicity. *International Journal of Dermatology*, **16**, 113.
- COOK, W.A., GIEVER, P.M., DINMAN, B.D. & MAGNUSON, J.H. (1971) Occupational acro-osteolysis. II. An industrial hygiene study. *Archives of Environmental Health*, **22**, 74.
- DEFALQUE, R.J. (1961) Pharmacology and toxicology of trichlorethylene: a critical review of the World literature. *Clinical Pharmacology and Therapeutics*, **2**, 1961.
- DINMAN, B.D., COOK, W.A., WHITEHOUSE, W.M., MAGNUSON, H.J. & DITCHECK, T. (1971) Occupational acro-osteolysis. I. An epidemiological study. *Archives of Environmental Health*, **22**, 61.
- GRANDJEAN, E., MUNCHINGER, R., TURRIAN, V., HAAS, P., KNOEPFEL, H.K. & ROSENEMUND, H. (1955) Investigations into the effects of exposure to trichlorethylene in mechanical engineering. *British Journal of Industrial Medicine*, **12**, 131.
- HARRISON, C.V. (1976) Lymph node diseases. In: *Recent Advances in Pathology*, No. 9 (Ed. by C.V.Harrison and K.Weinbren), p. 87. Churchill Livingstone, Edinburgh.
- LANGE, C.E., JUHE, S., STEIN, G. & VELTNAM, G. (1974) Vinyl chloride disease. *International Archives of Occupational Health*, **32**, 1.
- LOB, M. (1957) Les dangers du perchlorethylene. *Archiv für Gewerbepathologie und Gewerbehygiene*, **16**, 45.
- MESHKINPOUR, H., MYUNG, C.G. & KRAMER, L.S. (1977) A unique multisystemic syndrome of unknown origin. *Archives of Internal Medicine*, **137**, 1719.
- REINL, W. (1957) Sklerodermie durch Trichlorethylene—Einwirkung? *Zentralblatt für Arbeitsmedizin und Arbeitsschutz*, **7**, 58. Translated summary in *Bulletin of Hygiene* 1957, **32**, 678.
- SHIMPO, S. (1968) Solitary plasmacytoma with polyneuritis and endocrine disturbances. *Japanese Journal of Clinical Medicine*, **26**, 2444.
- SIMMONS, F., FELDMAN, B. & GERETY, D. (1972) Granulomatous hepatitis in a patient receiving allopurinol. *Gastroenterology*, **62**, 101.
- SMITH, G.F. (1966) Trichlorethylene; a review. *British Journal of Industrial Medicine*, **23**, 249.
- SPARROW, G.P. (1977) A connective tissue disorder similar to vinyl chloride disease in a patient exposed to perchlorethylene. *Clinical and Experimental Dermatology*, **2**, 17.
- WALKER, A.E. (1975) A preliminary report of a vascular abnormality occurring in men engaged in the manufacture of polyvinyl chloride. *British Journal of Dermatology*, **93**, Suppl. **11**, 22.
- WALKER, A.E. (1976) Clinical aspects of vinyl chloride disease: skin. *Proceedings of the Royal Society of Medicine*, **69**, 286.

Co-existence of pemphigus, anti-skeletal muscle antibody and a retroperitoneal paraganglioma

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SUMMARY

Occasional reports have appeared in the literature describing a relationship between pemphigus vulgaris and malignancy (Krain & Bierman, 1974; Krain, 1974; Tagami *et al.*, 1976). Carcinoma of the ovary, stomach, breast, endometrium and bronchus have all been associated with pemphigus (Krain, 1974). The association between pemphigus and thymoma is well known (Stillmand & Baer, 1972). Pemphigus has also been described in connection with various lymphoproliferative malignancies (Naysmith & Hancock, 1976).

The purpose of this paper is to describe a patient who suffered from a rare retroperitoneal tumour in association with pemphigus vulgaris. His serum contained a high titre of anti-skeletal muscle antibody, although he showed no clinical manifestations of myasthenia gravis. He also had immunological manifestations suggestive of lupus erythematosus.

CASE REPORT

This 62-year-old white male presented in January, 1977 to the Waveney Hospital, Ballymena with a 6-week history of cough and hoarseness, shortness of breath and weight loss of 3-4 stone over the past year.

On examination there was a tender mass in the left hypochondrium five finger-breadths below the costal margin. This was thought to be spleen. No other abnormality was detected. A chest X-ray was normal, as was the ESR. Liver function tests, blood urea, a bone marrow aspirate and trephine biopsy were normal.

In February 1977 some small glands were noted in the axilla; a biopsy showed non-specific reactive changes.

He was transferred to the Royal Victoria Hospital, Belfast in March 1977. At this stage he had a profuse, red, papular, itchy rash, some of the lesions developing small blisters. It commenced on the shoulders and then spread to the upper and lower limbs and the trunk. There were herpes-like lesions around the mouth and small superficial ulcers on the tongue and posterior pharynx. A biopsy

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of skin revealed features suggestive of pemphigus vulgaris. Immunofluorescence studies of the biopsy and also of the patient's serum were performed. A culture of the skin lesions grew *Staphylococcus aureus* coagulase positive.

The skin was treated with Polyfax ointment for 3 days and then with Betnovate cream; by this time the rash had become generalized, vesicular and even bullous (up to 2 cm in diameter). Following the biopsy report the treatment was changed to prednisone 40 mg daily, oral Fucidin and sulphadimidine. This resulted in considerable improvement, with disappearance of the blisters and alleviation of the itch.

A lymphogram and IVP were performed and these revealed a large retroperitoneal mass displacing the left kidney and ureter superiorly and anteriorly.

A laparotomy was performed and this disclosed a huge, vascular retroperitoneal tumour arising to the left of the mesentery and below the transverse colon. The spleen was displaced superiorly, the left kidney laterally, and the aorta to the right. The tumour was considered inoperable but a biopsy was taken.

Post-operatively the patient's condition improved somewhat and he was discharged, to be followed as an out-patient.

Unfortunately, the patient died at home and an autopsy was not performed.

MATERIALS AND METHODS

Half of the skin biopsy was fixed in Bouin's fluid and subsequently embedded in paraffin wax; 6 μ m paraffin sections were stained with haematoxylin and eosin (H & E). The remainder was rapidly frozen in isopentane (2-methyl butane)—precooled with liquid nitrogen. Cryostat sections 4 μ m thick were treated by standard immunofluorescence techniques, as were repeat serum samples (McKee & Sandford, 1978).

The biopsy of the tumour was fixed in mercuric formol solution and subsequently embedded in wax. Paraffin sections 6 μ m thick were stained with haematoxylin and eosin, Foot's reticulin stain, Weigert's elastic tissue stain, Masson-Fontana for melanin, Perl's Prussian blue for iron, toluidine blue and brilliant cresyl violet for Nissl substance.

RESULTS

The tumour consisted of uniform cells with a central vesicular nucleus containing an intensely eosinophilic nucleolus. The cytoplasm was eosinophilic and granular, and cell membranes were quite indistinct (Fig. 1). There was little nuclear pleomorphism and mitotic figures were infrequent. Scattered through the tumour were collections of lymphocytes. There was a rich vascular pattern. A reticulin stain showed the tumour cells to be arranged in clusters forming an alveolar pattern. Stains for iron, melanin and Nissl substance were negative. Interpretation of the histological features was difficult, but the structure was suggestive of a paraganglioma. Sections were sent to Professor R. Lattes at the College of Physicians and Surgeons at Columbia University, New York. He and his colleagues felt that the tumour most probably represented a paraganglioma but could not exclude with certainty the possibility of an 'epithelioid' type of haemangiopericytoma.

The skin biopsy was rather fragmented and showed pressure artefact but, nevertheless, a suprabasal vesicular cavity was identified containing acantholytic epidermal cells.

The results of direct immunofluorescence were as follows. Deposits of immunoglobulins (IgM and IgG) were found in the 'intercellular space' of the epidermis (Fig. 2). Immunoglobulins (IgG

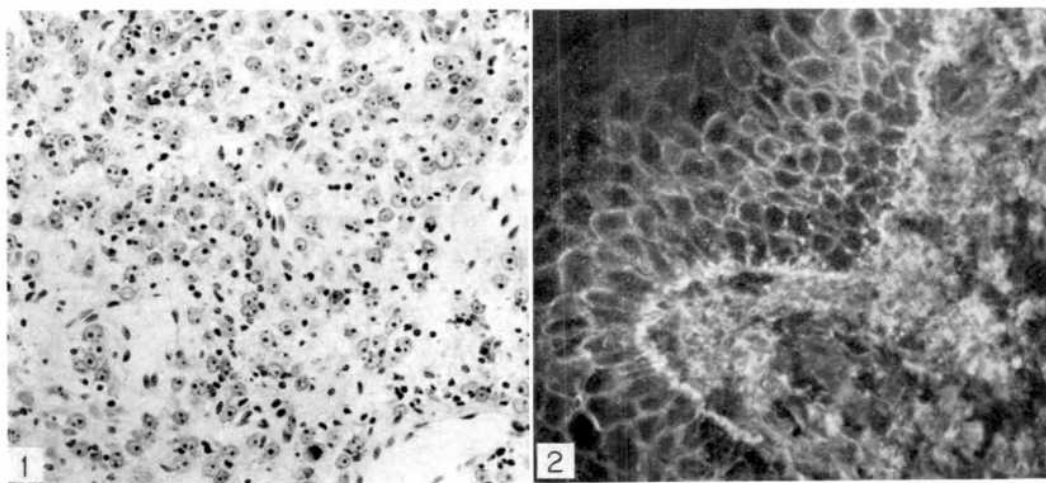


FIGURE 1. Histology of the tumour showing fairly large cells with regular nuclei and granular indistinct cytoplasm. There is an admixture of lymphocytes (H & E, $\times 100$).

FIGURE 2. Direct immunofluorescence of the patient's skin. Note prominent outlining of inter-cellular area (anti-IgM, $\times 250$).

and IgM) and complement C3 were noted as granular deposits at the epidermo-dermal junction (Fig. 3).

Examination of the patient's serum by the indirect immunofluorescent test revealed an anti-skeletal muscle antibody in a titre of 1:1280 (Fig. 4). No other antibodies, including pemphigus-type, were detected.

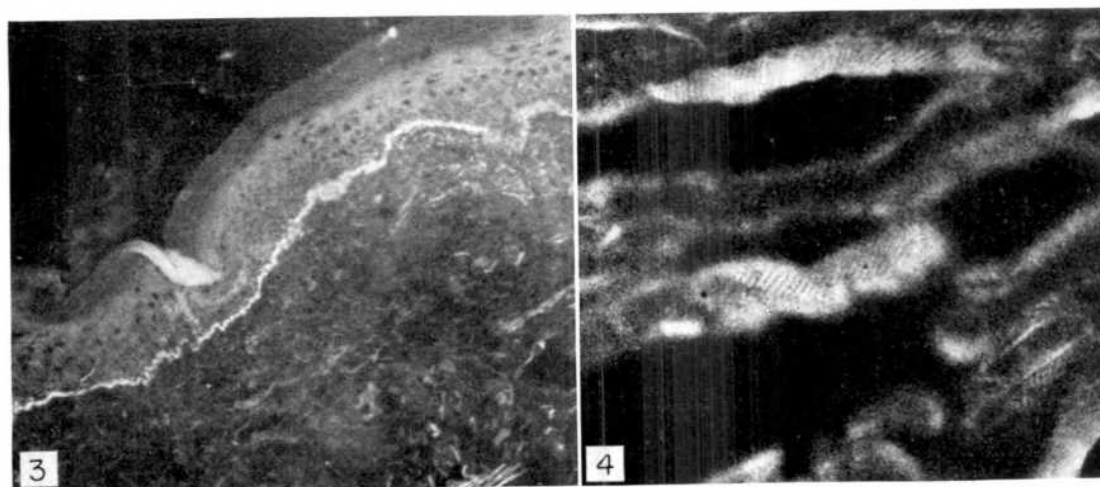


FIGURE 3. Direct immunofluorescence of the patient's skin. A brilliant, almost continuous granular fluorescence is seen at the basement membrane region (anti-C₃, $\times 100$).

FIGURE 4. The indirect immunofluorescent technique using human skeletal muscle as substrate. Note fluorescence of cross striations (anti-IgG, $\times 250$).

DISCUSSION

Beutner and his colleagues (1968) described the first patient with myasthenia gravis and associated pemphigus. Noguchi & Hishitani (1976), on reviewing the literature, collected only 10 reported cases of pemphigus associated with myasthenia gravis. They added the eleventh.

Krain (1974) and Krain & Bierman (1974) reviewed the literature concerning the association of pemphigus with various malignant tumours. Of 59 reported cases they accepted only 26 as authentic. Of these, there was wide variation in the type of malignancy. Carcinoma of ovary, stomach, breast, endometrium, bladder, oesophagus and bronchus were mentioned, as was choriocarcinoma and Kaposi sarcoma. Eight of the cases, however, were associated with a concurrent thymoma.

This patient was diagnosed clinically as suffering from pemphigus vulgaris. This was confirmed by histology and immunofluorescence examination of a skin biopsy. Repeated serum studies failed to elicit a pemphigus antibody. He also had a retroperitoneal tumour thought to be a paraganglioma. Examination of his serum revealed a high titre anti-skeletal muscle antibody and immunofluorescent examination of his skin biopsy demonstrated features suggestive of lupus erythematosus.

This, therefore, possibly represents a new variant of the pemphigus/myasthenia gravis group—paraganglioma associated with pemphigus and anti-skeletal muscle antibody with immunofluorescence manifestations of lupus erythematosus.

Anti-skeletal muscle antibody has been known to be associated with myasthenia gravis for some time. Strauss *et al.* (1965) investigated the incidence of anti-skeletal muscle antibody in myasthenia gravis patients and controls. They showed that in some controls a titre of up to 1:30 was found. Accepting a titre of 1:60 or greater as significant, they showed that the antibody was found mainly in patients with myasthenia gravis, although on occasion it was also detected in patients with inflammatory or wasting diseases of skeletal muscle.

In this case, the patient did not show any clinical features of myasthenia gravis or evidence of other muscle disease; it may well be, however, that if the patient had survived for a sufficiently long period of time, typical clinical symptomatology would have developed.

A variety of mechanisms have been suggested to explain the association between pemphigus and internal malignancy (Krain, 1974). These include (1) the secretion of antibodies by the tumour; (2) the sharing of common antigenic determinants by the tumour, the intercellular space of squamous epithelium and skeletal muscle; and (3) a primary immunological disturbance leading to the development of the tumour and the production of the various antibodies.

This paper, thus, adds another case linking pemphigus with internal malignancy and lends weight to the suggestion that patients presenting with pemphigus should be investigated thoroughly for an underlying neoplasm.

ACKNOWLEDGMENTS

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REFERENCES

- BEUTNER, E.H., CHORZELSKI, T.P., HALE, W.L. & JORDON, R.E. (1968) Autoimmunity in concurrent myasthenia gravis and pemphigus erythematosus. *Journal of the American Medical Association*, **203**, 845.
KRAIN, L.S. (1974) The association of pemphigus with thymoma or malignancy: a critical review. *British Journal of Dermatology*, **90**, 397.
KRAIN, L.S. & BIERMAN, S.M. (1974) Pemphigus vulgaris and internal malignancy. *Cancer*, **33**, 1091.

- LATTES, R. (1978) Personal communication.
- McKEE, P.H. & SANDFORD, J.C. (1978) The use of immunofluorescent techniques in dermatology. *Ulster Medical Journal*, **47**, in press.
- NAYSMITH, A. & HANCOCK, B.W. (1976) Hodgkin's disease and pemphigus. *British Journal of Dermatology*, **94**, 695.
- NOGUCHI, S. & NISHITANI, H. (1976) Immunologic studies of a case of myasthenia gravis associated with pemphigus vulgaris after thymectomy. *Neurology*, **26**, 1075.
- STILLMAN, M.A. & BAER, R.I. (1972) Pemphigus and thymoma. *Acta dermato-venereologica*, **52**, 393.
- STRAUSS, A.J.L., VANDER GELD, H.R.W., KEMP, P.G., EXUM, E.D. & GOODMAN, H.D. (1965) Immunological concomitants of myasthenia gravis. *Annals of the New York Academy of Science*, **124**, 744.
- TAGAMI, H., IMAMURA, S., NOGUCHI, S. & NISHITANI, H. (1976) Co-existence of peculiar pemphigus, myasthenia gravis and malignant thymoma. *Dermatologica*, **152**, 181.

Bullous pemphigoid and primary biliary cirrhosis

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SUMMARY

The occurrence of multiple autoimmune conditions in an individual and in families is well known. We wish to report the simultaneous occurrence of bullous pemphigoid and primary biliary cirrhosis in an individual who also suffered from vitiligo. The association adds to the documentation of bullous pemphigoid co-existing with other autoimmune disorders.

CASE REPORT

A 70-year-old man was seen in November 1977 with an itchy, blistering eruption of 1 month's duration. Tense and haemorrhagic blisters involved the trunk and the proximal parts of the limbs. Vitiligo was present on the neck and the flexor aspects of his wrists. He was not jaundiced and was itchy only in areas involved by blisters. The liver was palpable 4 cm below the subcostal margin. The spleen was not palpable.

A clinical diagnosis of bullous pemphigoid was made and histology showed typical sub-epidermal bullae. We could not demonstrate either IgG or C3 BMZ antibody by indirect immunofluorescence. We were unable to perform direct immunofluorescence at the time of his eruption but it was negative when healed skin was later tested.

Liver function tests during November 1977 were consistently abnormal: bilirubin 6 $\mu\text{mol/l}$ (N.R. up to 17); alkaline phosphatase 566 iu/l (N.R. 30–100); aspartate transaminase (AST) 59 iu/l (N.R. 9–27); alanine transaminase (ALT) 64 iu/l (N.R. 2–24); 5-nucleotidase 59.8 iu/l (N.R. 6–17); γ -glutamyl transpeptidase 216 iu/l (N.R. 6–32). The serum cholesterol and triglycerides were normal. Autoantibodies to mitochondria were positive in a titre of more than 1 in 400. IgM was elevated at 4.4 g/l (N.R. 0.47–1.70). Latex (R.A.) and antinuclear factor were absent. Serum thyroxine, folate and vitamin B₁₂ were normal. A liver biopsy showed several broad bands of fibrosis in which lymphoid follicles were present. The portal tracts showed infiltration with plasma cells and lymphocytes surrounding small bile ducts. The changes were diagnostic of primary biliary cirrhosis. No specific direct immunofluorescence was seen.

The bullous pemphigoid was treated with prednisolone 30 mg per day with gradual reduction to a maintenance dosage of 10 mg per day. During the period of steroid therapy his liver function tests improved, and 1 month after starting treatment the liver function tests (bilirubin, alkaline phosphatase, AST and ALT) were normal. Three months after starting steroids his liver has remained firm and

palpable 4 cm below the costal margin. The family history contained no evidence of autoimmune disease apart from a sister with seropositive rheumatoid arthritis and a positive antinuclear factor.

DISCUSSION

The presence of autoantibodies against basement membrane in 70% of instances of bullous pemphigoid suggests that it may be an autoimmune disease (Nieboer, Roeleveld & Kalsbeek, 1978). A relationship between bullous pemphigoid and other autoimmune disease has been infrequently reported. It has been reported in association with rheumatoid arthritis (Lillicrap, 1963; Salo & Räsänen, 1972) systemic lupus erythematosus (Jordon *et al.*, 1969), polymyositis (Peck & Lefkovits, 1966), and pernicious anaemia (Muller *et al.*, 1971; Obasi & Savin, 1977). Waltman & Yarian (1974) described the presence of antinuclear factor in instances of ocular pemphigoid, while Muller *et al.* (1971) documented two patients with pemphigoid and autoantibodies against gastric parietal cells and thyroid cytoplasm.

In a retrospective study of 20 patients with bullous pemphigoid seen at the Norfolk and Norwich Hospital over the last 13 years, in whom auto-antibodies were studied, 2 patients were found to have positive antibodies against thyroid cytoplasm, one of whom subsequently developed myxoedema. One patient had a positive antinuclear factor, and one patient had pre-existing diabetes. One patient's pemphigoid was associated with vitiligo. We can find no reference in the literature to the co-existence of vitiligo and either primary biliary cirrhosis or bullous pemphigoid. Vitiligo itself is often associated with other autoimmune conditions (Brostoff, Bor & Feiwel, 1969), and while its aetiology may have an autoimmune basis (Editorial, 1971), antimelanocyte antibodies have only rarely been found (Langhof, Feuerstein & Schabinski, 1965; Hertz *et al.*, 1977).

There is increasing evidence that primary biliary cirrhosis may have an autoimmune basis (Doniach *et al.*, 1970; Doniach, 1972) or represent an immune complex disease (Thomas, Potter & Sherlock, 1977; Wands *et al.*, 1978). An antibody reacting with mitochondria was described in the sera of patients with primary biliary cirrhosis by Walker *et al.* (1965), and subsequently this antibody has been found in approximately 90% of instances of the disease (Wright, 1975). Primary biliary cirrhosis may co-exist with a wide range of other autoimmune conditions, and has been reported with Sjögren's syndrome and renal tubular acidosis (Goldring, Smith & Williams, 1973), rheumatoid arthritis (Sherlock & Scheuer, 1973; Child, Mathews & Thompson, 1977; Mills, MacSween & Watkinson, 1977), systemic sclerosis (Murray-Lyon *et al.*, 1970; Reynolds *et al.*, 1971), cutaneous capillaritis and membranous glomerulonephritis (Rai *et al.*, 1977), autoimmune thyroiditis (Walker *et al.*, 1972), autoimmune adrenal failure (Sherlock & Scheuer, 1973) and coeliac disease (Logan, *et al.*, 1978). While the high incidence of antimitochondrial antibodies in primary biliary cirrhosis is of diagnostic significance, the autoantibody may also be found in a much lower percentage in non-hepatic autoimmune diseases. In thyrotoxicosis, Hashimoto's thyroiditis, pernicious anaemia, Addison's disease and myasthenia gravis, 2-3% positive results were obtained compared with less than 1% of normal controls (Doniach, 1972). We have not previously encountered the antibody in bullous pemphigoid. Primary biliary cirrhosis usually presents with pruritus, jaundice and malabsorption, but asymptomatic cases have been reported (Fox, Scheuer & Sherlock, 1973; Long, Scheuer & Sherlock, 1977). The present case appears to be a further example. The occurrence of primary biliary cirrhosis in a male in contrast to its usual female preponderance suggests that its association in this patient with bullous pemphigoid may be more than fortuitous. The return to normality of his liver function tests while on systemic steroids is of interest. While failure to respond to steroids has been used as a diagnostic aid in distinguishing primary biliary cirrhosis from chronic active hepatitis (Geubel, Baggenstoss & Summer-

skill, 1976), biochemical improvement with steroids has been reported in the absence of parallel histological recovery (MacKay, 1958).

REFERENCES

- BROSTOFF, J., BOR, S. & FEIWEL, M. (1969) Autoantibodies in patients with vitiligo. *Lancet*, **ii**, 177.
- CHILD, D.L., MATHEWS, J.A. & THOMPSON, R.P.H. (1977) Arthritis and primary biliary cirrhosis. *British Medical Journal*, **ii**, 557.
- DONIACH, D., WALKER, J.G., ROITT, I.M. & BERG, P.A. (1970) 'Autoallergic' hepatitis. *New England Journal of Medicine*, **282**, 86.
- DONIACH, D. (1972) Autoimmune aspects of liver disease. *British Medical Bulletin*, **28**, 145.
- EDITORIAL (1971) Aetiology of vitiligo. *Lancet*, **ii**, 1298.
- FOX, R.A., SCHEUER, P.J. & SHERLOCK, S. (1973) Asymptomatic primary biliary cirrhosis. *Gut*, **14**, 444.
- GEUBEL, A.P., BAGGENSTOSS, A.H. & SUMMERSKILL, W.H.J. (1976) Responses to treatment can differentiate chronic active liver disease with cholangitic features from the primary biliary cirrhosis syndrome. *Gastroenterology*, **71**, 444.
- GOLDING, P.L., SMITH, M. & WILLIAMS, R. (1973) Multisystem involvement in chronic liver disease. *American Journal of Medicine*, **55**, 772.
- HERTZ, K.C., GAZZE, L.A., KIRKPATRICK, C.H. & KATZ, S.I. (1977) Autoimmune vitiligo: detection of antibodies to melanin-producing cells. *New England Journal of Medicine*, **297**, 634.
- JORDON, R.E., MULLER, S.A., HALE, W.L. & BEUTNER, E.H. (1969) Bullous pemphigoid associated with systemic lupus erythematosus. *Archives of Dermatology*, **99**, 17.
- LANGHOF, VON M., FEUERSTEIN, M. & SCHABINSKI, G. (1965) Me aninatikörperbildung beim Vitiligo. *Hautarzt*, **16**, 209.
- LILLICRAP, D.A. (1963) Rheumatoid arthritis and pemphigoid. *Proceedings of the Royal Society of Medicine*, **56**, 921.
- LOGAN, R.F.A., FERGUSON, A., FINLAYSON, N.D.C. & WEIR, D.G. (1978) Primary biliary cirrhosis and coeliac disease. *Lancet*, **i**, 230.
- LONG, R.G., SCHEUER, P.J. & SHERLOCK, S. (1977) The presentation and course of asymptomatic primary biliary cirrhosis. *Gastroenterology*, **72**, 1204.
- MACKAY, I.R. (1958) Primary biliary cirrhosis showing a high titer of autoantibody. *New England Journal of Medicine*, **258**, 185.
- MILLS, P., MACSWEEN, R.N.M. & WATKINSON, G. (1977) Arthritis and primary biliary cirrhosis. *British Medical Journal*, **ii**, 1224.
- MULLER, H.K., SUTHERLAND, R.C., JAMIESON, W.J. & DE BOER, W.G.R.M. (1971) Autoantibodies in bullous pemphigoid. *Medical Journal of Australia*, **1**, 429.
- MURRAY-LYON, I.M., THOMPSON, R.P.H., ANSELL, I.D. & WILLIAMS, R. (1970) Scleroderma and primary biliary cirrhosis. *British Medical Journal*, **iii**, 258.
- NIEBOER, C., ROEVELD, C.G. & KALSBECK, G.L. (1978) Localised chronic pemphigoid *Dermatologica*, **156**, 24.
- OBASI, O.E. & SAVIN, J.A. (1977) Pemphigoid and pernicious anaemia. *British Medical Journal*, **ii**, 1458.
- PECK, S.M. & LEFKOVITS, A.M. (1966) Bullous pemphigoid, polymyositis and co-existing contact dermatitis. *Archives of Dermatology*, **94**, 672.
- RAI, G.S., HAMLYN, A.N., DAHL, M.G.C., MORLEY, A.R. & WILKINSON, R. (1977) Primary biliary cirrhosis, cutaneous capillaritis and IgM-associated membranous glomerulonephritis. *British Medical Journal*, **i**, 817.
- REYNOLDS, T.B., DENISON, E.K., FRANKL, H.D., LIEBERMAN, F.L. & PETERS, R.L. (1971) Primary biliary cirrhosis with scleroderma, Raynaud's phenomenon and telangiectasia. *American Journal of Medicine*, **50**, 302.
- SALO, O.P. & RASANEN, J.A. (1972) Pemphigoid and rheumatoid arthritis: a clinical and immunological study. *Annals of Clinical Research*, **4**, 173.
- SHERLOCK, S. & SCHEUER, P.J. (1973) The presentation and diagnosis of 100 patients with primary biliary cirrhosis. *New England Journal of Medicine*, **289**, 674.
- THOMAS, H.C., POTTER, B.J. & SHERLOCK, S. (1977) Is primary biliary cirrhosis an immune complex disease? *Lancet*, **ii**, 1261.
- WALKER, J.G., DONIACH, D., ROITT, I.M. & SHERLOCK, S. (1965) Serological tests in diagnosis of primary biliary cirrhosis. *Lancet*, **i**, 872.
- WALKER, J.G., BATES, D., DONIACH, D., BALL, P.A.J. & SHERLOCK, S. (1972) Chronic liver disease and mitochondrial antibodies: a family study. *British Medical Journal*, **i**, 146.

- WALTMAN, S.R. & YARIAN, D. (1974) Circulating autc antibodies in ocular pemphigoid. *American Journal of Ophthalmology*, **77**, 891.
- WANDS, J.R., DIENSTAG, J.L., BHAN, A.K., FELLER, E.R. & ISSELBACHER, K.J. (1978) Circulating immune complexes and complement activation in primary biliary cirrhosis. *New England Journal of Medicine*, **298**, 233.
- WRIGHT, R. (1975) Immunology of liver disease. In: *Clinical Aspects of Immunology* (Ed. by P.G.H. Gell, R.R.A. Coombs and P.J. Lachmann), 3rd edn, p. 1285. Blackwell Scientific Publications, Oxford.

Epithelioid sarcoma—a case report

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SUMMARY

A case of epithelioid sarcoma is reported which clinically resembled sporotrichosis.

Epithelioid sarcoma is a recently described malignant tumour of connective tissue of the skin, subcutaneous tissues or fascial sheaths whose cells have an epithelioid appearance (Sanderson, 1972). It is a rare tumour. Enzinger (1970) reviewed 62 cases and up to 1975 the total number of cases recorded in the English language was 92 (Mackenzie, 1975).

The tumour is more common in males with a male to female ratio of 2.4 to 1 and the average age of onset was 33 years. Almost all the tumours originated in the extremities, the majority in the upper limb. Previous injury at the site of the first tumour was reported in 14 out of 62 of Enzinger's cases. The presenting sign was one or more slowly growing dermal nodules or subcutaneous lobular lesions. The tumour may be attached to deeper structures or may grow along a large nerve trunk. Chronic ulceration is a common occurrence and is very liable to be confused with a granuloma or a carcinoma. Histologically the tumour is composed of firm nodules 5–50 mm in diameter surrounded by fibrosis. The tendency to focal necrosis and the epithelioid nature of the cells with bright eosinophilic cytoplasm are the important features for diagnosis. It is considered a dangerous neoplasm due to frequent recurrences and distant metastatic spread. Unfortunately, it is often confused with many benign diseases both on clinical and histological features. We report here one case which fits with the typical description but which on clinical examination gave an erroneous impression of sporotrichosis.

CASE REPORT

A 23-year-old male farm labourer from one of the rural areas of Zambia presented with multiple painless nodular swellings on his right hand of 1 year duration. These started after an injury to his right palm with an old piece of broken glass while he was working in a field. The injury healed well initially but 2 months later small painless nodules appeared in the scar and ulcerated. Gradually similar lesions appeared around the wrist and extended up the arm to the axilla on the same side. Some of these nodules ulcerated and discharged small quantities of blood stained pus. The condition

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had been relatively painless throughout and his general health was unaffected. When he was seen in our clinic the fingers of his right hand were in a fixed semiflexed position. The small muscles of the hand were wasted and there was an inch long shallow ulcer in the mid palmar area with clean edges and without any discharge. There were multiple black nodular lesions of varying sizes from 5 mm to 5 cm extending from the wrist to the axilla. Some of them were in a linear and cord like form. The larger nodules were lobulated with a firm to hard consistency and fixed to the skin and the subcutaneous structures (Fig. 1). The nodular lesions around the wrist had deformed the joint but did not appear to be fixed to the bones. Three swellings were palpated in the axilla, one of them large (4 × 5 cm) and matted. All the other systems were normal and clinically the condition was diagnosed as sporotrichosis.



FIGURE 1. Multiple nodular lesions in a linear form along the forearm and upperarm.

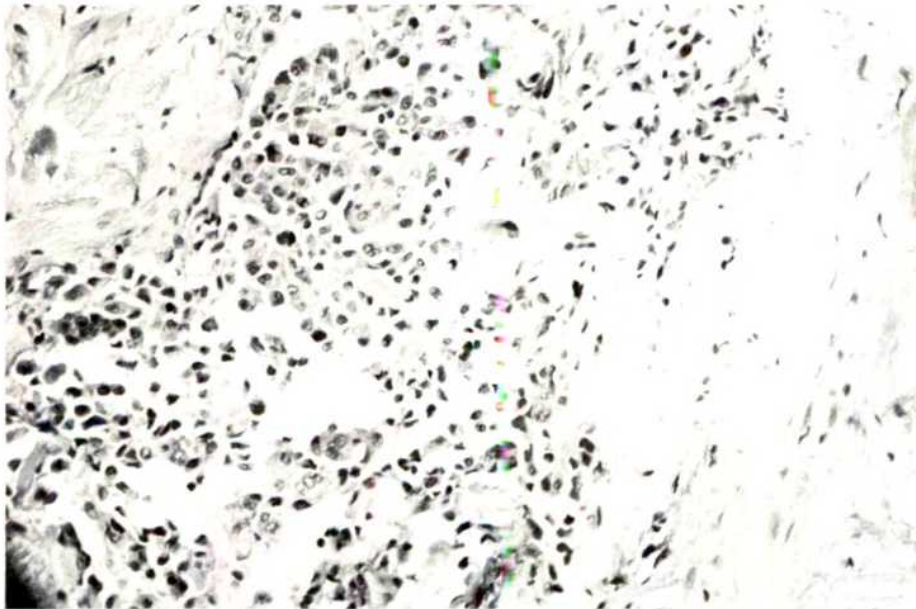


FIGURE 2. Typical island of epithelioid tumour cells surrounded by fibrocollagenous tissue (× 232).

All routine investigations including a chest X-ray proved to be normal. X-rays of his right hand revealed no bony involvement but a soft tissue mass displaced the lower end of the ulna without actual infiltration of the bone. Repeated cultures of the tumour discharge and aspirates, contrary to our expectation, failed to grow any fungus. Biopsy of a nodule revealed clusters of anaplastic epithelioid tumour cells surrounded by fibrocollagenous proliferation in the subcutis and

lower dermis. The tumour cells were round or polygonal and a few spindle shaped with a bright eosinophilic cytoplasm (Fig. 2). There were also collections of chronic inflammatory cells around the tumour islands. The condition was diagnosed histologically as epithelioid sarcoma. Unfortunately, the patient absconded from the wards before we arrived at this diagnosis and no further follow up could be undertaken.

DISCUSSION

Although this neoplasm arises in the skin, only one other case so far was reported presented as a dermatological problem (Saxe & Botha, 1977). We also feel that the mistaken clinical diagnosis of sporotrichosis in our patient should sound a warning that this dangerous tumour can give a false clinical impression.

ACKNOWLEDGMENTS

We wish to thank Mr Ansary of Medical Illustration Unit, University of Zambia for the photographs.

REFERENCES

- ENZINGER, F.M. (1970) Epithelioid sarcoma. *Cancer, N.Y.*, **26**, 1029.
MACKENZIE, D.H. (1975) Miscellaneous soft tissue sarcomas. In: *Recent Advances in Pathology* (Ed. by C.V. Harrison and K. Weinbren), p. 206. Churchill Livingstone, Edinburgh.
SANDERSON, K.V. (1972) Tumours of the skin. In: *Textbook of Dermatology* (Ed. by A.Rook, D.S. Wilkinson and F.J.G.Ebling), 2nd edn. Vol. 2, p. 1991. Blackwell Scientific Publications, Oxford.
SAXE, N. & BOTHA, J.B.C. (1977) Epithelioid sarcoma: a distinctive clinical presentation. *Archives of Dermatology*, **113**, 1106.

Urticaria-like lesions associated with systemic lupus erythematosus: response to dapsone

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SUMMARY

A case of systemic lupus erythematosus is reported, presenting with urticaria-like lesions and complement deficiencies, which was completely controlled by dapsone.

Urticaria-like lesions may develop in patients with systemic lupus erythematosus (Braverman, 1967; Jansen & Cormane, 1967; Paver, 1971). Histologically the weals show vasculitis, and clinically they last for 2–4 days as opposed to some 8–24 h in ordinary chronic urticaria, and itching is not a feature.

Cases of vasculitis giving rise to urticarial weals have recently been described in association with deficiency in various parts of the complement chain (McDuffie *et al.*, 1973; Sissons *et al.*, 1974; Soter, 1977).

Dapsone has been used for many years in the treatment of leprosy, dermatitis herpetiformis and more recently in some conditions in which vasculitis is present, such as rheumatoid arthritis, erythema elevatum diutinum (Vollum, 1968; Cream, Levene & Calnan, 1971), allergic vasculitis (Wells, 1969) and vasculitis associated with complement deficiency (Rendell, 1977). Its mode of action is unknown. It probably has an effect on the complement pathway (Millikan & Conway, 1976; Thompson & Souhami, 1975) but this has not been confirmed (Katz *et al.*, 1976).

CASE REPORT

A 53-year-old white nursing auxiliary presented in 1976 with an 18-month history of recurrent, non-itchy urticarial weals each lasting up to 3–4 days and, at times, angio-oedema of eyelids and upper lip. She had never been free from lesions since the onset. For 6 months she also had recurrent bouts of pain and redness of the eyes every 2–3 weeks and had occasional joint pains—particularly in the limbs. Six months after she was first seen she developed two discrete, red, scaly lesions on the upper cheeks which were, clinically, consistent with discoid lupus erythematosus.

Plasma viscosity, blood count, liver function tests, immunoglobulins and creatinine clearance were normal, but there were low levels of C1, C2 and C3, and absence of C1q and CH₅₀. The auto-immune

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profile was normal except for the ANF which was present at 1:40; the DNA binding was 25%. The histology of weals showed a mild perivascular lymphocytic infiltrate which was not diagnostic, but the immunofluorescence of lesional skin showed IgM in the basement membrane zone. Immunofluorescence of non-involved, light-exposed skin from the wrist showed all the features of systemic lupus erythematosus with granular deposits of IgG, IgA and IgM and complement in the basement membrane zone, and fibrin in the vessels (Dr J.W.B. Bradfield).

All the lesions cleared with prednisone 30 mg daily, but she developed oedema and hypertension (BP 220/120 mm Hg) and gained weight. When the dose was lowered to 20 mg daily the rash recurred. Azathioprine 200 mg daily for 6 weeks was ineffective and produced nausea. The hypertension and oedema settled a few weeks after stopping the steroid.

A trial of dapsone (150 mg daily) produced dramatic clearing of her skin lesions within 2-3 days and she remained free of rash and eye symptoms until the dapsone was stopped. Within 3 days there was recurrence of the rash, but this cleared again within a few days of re-starting dapsone, and she then remained clear. She attended the clinical meeting at the British Association of Dermatologists' meeting in Bristol in July 1977 and kindly left off the dapsone for a week prior to this. She then developed a large number of weals, which once again cleared within a few days of re-starting dapsone. The patient was last seen in May 1978 and had remained completely clear of weals and other symptoms whilst taking dapsone, but if this was discontinued weals still developed after 2-3 days. The C1, C2 and CH₅₀ levels approached normal values after she had taken dapsone for 9 months, but the C3 level remained low.

COMMENT

There seems little doubt that the patient has systemic lupus erythematosus presenting with an urticaria-like eruption. The effect of dapsone on the skin lesions and on the recurrent eye and joint symptoms was quite dramatic.

It would be very interesting to know whether dapsone will affect vasculitis in organs other than the skin, and be a worth-while treatment in some cases of systemic lupus erythematosus.

ACKNOWLEDGMENTS

We thank Professor P. Lachmann for undertaking the complement study and Dr Doris Fletcher for kindly referring the patient to us.

REFERENCES

- BRAVERMAN, I.M. (1967) Urticaria as a sign of internal disease. *Postgraduate Medicine*, **41**, 450.
- CREAM, J.J., LEVENE, G.M. & CALNAN, C.D. (1971) Erythema elevatum diutinum. An unusual reaction to Streptococcal antigen and response to dapsone. *British Journal of Dermatology*, **84**, 393.
- JANSEN, L.H. & CORMANE, R.H. (1967) Urticaria bij lupus erythematosus. *Netherlands Tijdschrift Voor Geneeskunde*, **111**, 2160.
- KATZ, S.I., HERTZ, K.C., CRAWFORD, P.S., GAZZE, L.A., FRANK, M.M. & LAWLEY, T.J. (1976) Effect of sulfones on complement deposition in dermatitis herpetiformis and on complement-mediated guinea-pig reactions. *Journal of Investigative Dermatology*, **67**, 688.
- MCDUFFIE, F.C., SAMS, W.M., MALDONADO, J.E., ANDREINI, P.H., CONN, D.L. & SAMAYOA, E.A. (1973) Hypocomplementemia with cutaneous vasculitis and arthritis. Possible immune complex syndrome. *Mayo Clinic Proceedings*, **48**, 340.
- MILLIKAN, L.E. & CONWAY, F.R. (1976) Effect of drugs on the Pillner Pathway-Dapsone (abstract). *Journal of Investigative Dermatology*, **62**, 541.

- PAVER, W.K. (1971) Discoid and subacute lupus erythematosus associated with urticaria. *Australasian Journal of Dermatology*, **12**, 113.
- RENDELL, J.R.S. (1977) Vasculitis with probable familial C2 deficiency. *Proceedings of the Royal Society of Medicine*, **70**, 733.
- SISSONS, J.G.P., WILLIAMS, D.G., PETERS, D.K. & BOULTON-JONES, J.M. (1974) Skin lesions, angio-oedema and hypocomplementaemia. *Lancet*, **ii**, 1350.
- SOTER, N.A. (1977) Chronic urticaria as a manifestation of necrotizing venulitis. *New England Journal of Medicine*, **296**, 1440.
- THOMPSON, D.M. & SOUHAMI, R. (1975) Suppression of the Arthus reaction in the guinea-pig by dapsone (abstract). *Proceedings of the Royal Society of Medicine*, **68**, 273.
- VOLLUM, D.I. (1968) Erythema elevatum diutinum: vesicular lesions and sulphone response. *British Journal of Dermatology*, **80**, 178.
- WELLS, G.C. (1969) Allergic vasculitis (Tri-symptom of Gougerot). Treated with dapsone. *Proceedings of the Royal Society of Medicine*, **62**, 665.

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Obituaries

BARNEY DAVID USHER

Dr Barney Usher died on 14 July 1978. He had been an Honorary Member of The British Association of Dermatologists since 1967.

PROFESSOR K. KIRÁLY

Professor K. Király, Professor of Dermatology in Budapest, died suddenly on 18 May 1978.

He had been nominated for election as an Honorary Foreign Member of The British Association of Dermatologists in July 1978.

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Infrared radiation as a carcinogenic agent

SIR. In his outstanding article, 'A concept of diffuse actinic arteritis,' O'Brien suggests that infrared radiation from the sun may have an important aetiological significance in arteritis of the temporal artery and in polymyalgia rheumatica (O'Brien, 1978). He notes that infrared radiation may be aetiological in erythema ab igne and that studies show its penetration into the epidermis and dermis. This concept of infrared body surface damage might be extended further.

The concept of electromagnetic wave-induced carcinogenesis is at present most active with regard to the spectrum immediately below visible light, that is, between 320 and 400 nm, the so-called ultraviolet (UV) A spectrum. The ability of UV-A to induce skin cancer either directly or in combination with UV-B, the major carcinogenic range, is under considerable evaluation (Willis & Cylus, 1977). In reviewing the subject of carcinogenesis induced by UV and other electromagnetic waves (Schwartz & Klein, 1978; Klein & Schwartz, 1978), I have noted a deficit of work in consideration of the spectrum immediately above visible light, that is, the infrared spectrum of 700 nm and above to 1,000,000 nm. Infrared radiation has been implicated in erythema ab igne (Finlayson, Sams & Smith, 1966). Erythema ab igne has been shown to predispose to skin cancer in Ireland (Cross, 1967). Infrared radiation represents about 40% of solar output (Koller, 1965). A 1964 study showed that acute exposure to infrared (IR) exaggerated UV radiation-induced erythema in mice and that chronic IR exposure increased both the incidence and the speed of development of cutaneous squamous cell carcinomas in these animals (Freeman & Knox, 1964). A more recent study suggested that IR may act synergistically with UV radiation to produce cutaneous DNA denaturation, although the role of this DNA change in carcinogenesis is unclear (Roth & London, 1977). Perhaps more significant is the finding that heat (IR) inactivates DNA repair enzymes (Corry *et al.*, 1977). It is said that since the relatively low energy of infrared photons may be insufficient to initiate photochemical reactions, IR is unlikely to have a role in cutaneous carcinogenesis (Pathak & Epstein, 1971); this merits re-evaluation.

Infrared radiation is appreciated by people as the sensation of heat. As one is struck by the warmth of the sun, one might contemplate the idea of infrared radiation-induced carcinogenesis.

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REFERENCES

- CORRY, P.M., ROBINSON, S. & GETZ, S. (1977) Hyperthermic effects on DNA repair mechanisms. *Radiology*, **123**, 475.
- CROSS, F. (1967) On a turf (peat) fire cancer: malignant change superimposed on erythema ab igne. *Proceedings of the Royal Society of Medicine*, **60**, 1307.
- FINLAYSON, G.R., SAMS, W.M., JR & SMITH, J.G. (1966) Erythema ab igne: a histopathologic study. *Journal of Investigative Dermatology*, **46**, 104.
- FREEMAN, R.G. & KNOX, J.M. (1964) Influence of temperature on ultraviolet injury. *Archives of Dermatology*, **89**, 858.
- KLEIN, E. & SCHWARTZ, R.A. (1978) Cancer and the skin. In: *Cancer Medicine* (Ed. by J.F. Holland and E. Frei, III), 2nd edn. Lea & Febiger, Philadelphia (in press).
- KOLLER, L.R. (1965) *Ultraviolet Radiation*. John Wiley & Sons, New York.
- O'BRIEN, J.P. (1978) A concept of diffuse actinic arteritis. *British Journal of Dermatology*, **98**, 1.
- PATHAK, M.A. & EPSTEIN, J.H. (1971) Normal and abnormal reaction of man to light. In: *Dermatology in General Medicine* (Ed. by T.B. Fitzpatrick, K.A. Arndt, W.C. Clark, A.L. Eisen, E.J. Van Scott and J.H. Vaughan). McGraw-Hill, New York.

- ROTH, D. & LONDON, M. (1977) Acridine probe study into synergistic DNA-denaturing action of heat and ultraviolet light in squamous cells. *Journal of Investigative Dermatology*, **69**, 368.
- SCHWARTZ, R.A. & KLEIN, E. (1978) Ultraviolet Light-induced carcinogenesis. In: *Cancer Medicine* (Ed. by J.F. Holland and E. Frei, III), 2nd edn. Lea & Febiger, Philadelphia (in press).
- WILLIS, I. & CYLUS, L. (1977) UVA erythema in skin: is it a sunburn? *Journal of Investigative Dermatology*, **68**, 128.

Erythema gyratum repens: spontaneous resolution in a healthy man

SIR, Barber *et al.* (1978) are the first to describe an association between erythema gyratum repens (E.G.R.) and a non-malignant process. Their article prompts the following report of EGR which settled spontaneously in an otherwise healthy man.

A man of 75 presented in February 1976 with a 3 month history of classical EGR. As EGR had been associated with an underlying malignancy in all previous patients he was admitted to the ward for thorough investigation. All the results were found to be normal including the following: Complete blood count, ESR 18 mm/h, urinalysis, faecal occult blood $\times 4$, throat swab, repeated liver function tests, urea and electrolytes, skeletal survey, X-ray chest, barium enema and meal, IVP. Topical and systemic therapy had no effect on the dermatosis and the patient was discharged from the ward for follow up as an outpatient.

In August 1977 the patient's EGR settled spontaneously. Immunology carried out at this time by Dr T.M.S.Reid, Department of Bacteriology, Aberdeen University, revealed normal numbers of T and B lymphocytes in the peripheral blood and normal T cell function as assessed by PHA transformation.

According to Skolnick & Mainman (1975) the onset of EGR may precede the discovery of malignancy by up to 15 months and is frequently the presenting sign.

Apart from the report of Barber *et al.* (1978) EGR has been reported to settle either after successful treatment of an underlying malignancy or spontaneously just prior to death, the latter attributed to loss of immunological competence (Skolnick & Mainman, 1975). The present patient is the first in whom EGR settled spontaneously in an otherwise healthy patient.

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REFERENCES

- BARBER, P.V., DOYLE, L., VICKERS, D.M. & HUBBAED, H. (1978) Erythema gyratum repens with pulmonary tuberculosis. *British Journal of Dermatology*, **98**, 465.
- SKOLNICK, M. & MAINMAN, E.R. (1975) Erythema gyratum repens with metastatic adenocarcinoma. *Archives of Dermatology*, **111**, 227.

Theses

Theses received from L'Université Claude Bernard, Lyon 1, France. (Professor J. Thivolet).

- COUSSA, MARIE-SYLVIE (1977) Traitement du psoriasis par photochimiothérapie orale (à propos de 100 observations).
- FAURE, MICHEL (1978) Chimiotaxie du polynucléaire neutrophile. Physiologie. Aspects pathologiques. Sa place dans les processus de la phagocytose (à propos d'une observation d'association de déficit de la chimiotaxie et d'hypergammaglobulinémie E sérique).
- FRIONNET, MARYSE (1977) Immunologie du pemphigus: Intérêt diagnostique et pronostique.
- SOUTEYRAND, PIERRE (1977) Lymphomes cutanés malins: Apport des techniques immunologiques et ultrastructurales.

Book Reviews

Scabies and Pediculosis. By M. ORKIN, H.I. MAIBACH, L.C. PARISH and R.M. SCHWARTZMAN (1977) Philadelphia: J.B. Lippincott. Pp. 203. Col. plates. B/W illus. Price £8.00.

In May 1976 an International Conference on Scabies and Pediculosis was held in Minneapolis; this book contains a series of essays by the participants.

There are entertaining chapters on the history of the insects by Parish; Mellanby and Busvine provide elegant contributions on the biology of the parasites. Opinions on the epidemiology and clinical aspects are offered from countries as varied as Norway and Venezuela. The result is a book of 200 pages which covers the subjects very thoroughly and, as might be expected, repetitiously. Most chapters contain a list of references sufficiently comprehensive to make it a useful source book.

The epidemiology and its relationship to sexual promiscuity or lack of immunity in the young is repeatedly discussed. The answer—both—is neatly given in the verse by Mellanby:

Recondite research on Sarcoptes
Has revealed that infections begin
At home with your wives and your children
Or when you are living in sin. . .

Several authors refer to skin scrapings as a means of diagnosis and only the historical Parish and the experienced Lomholt describe how to remove an acarus with a pin. Fashions in therapy vary from country to country but I was surprised to find sulphur ointment still widely used.

The conference does not appear to have produced any new knowledge and speculations as to the future are most relevant in pediculosis where resistance to organochlorine and organophosphorus compounds is increasing, but even here no great hope of advance could be proffered.

This is a useful book of reference for any dermatological library.

RONALD CHURCH

The Ichthyoses. Edited by R. MARKS and P.J. DYKES (1978) Lancaster: MTP. Pp. xi + 205. Price £11.95.

This volume consists of the proceedings of the Second Annual clinically orientated Symposium of the

European Society for Dermatological Research. The symposium, organized by Dr Ronald Marks, took place in Cardiff, Wales. The thirty-nine contributors were drawn from six countries; nineteen from the United Kingdom, seven from Italy, five each from Germany and the United States, two from France and one from Sweden. The papers were arranged in three sections: pathogenic aspects, clinical and genetic aspects, and treatment. Together they present an invaluable review of a fascinatingly complex group of disorders, with emphasis on those areas of research in which useful progress is being made. This book can therefore be strongly recommended both to clinicians and to investigators. Dr Marks and his colleagues deserve our gratitude for making so successfully the very considerable effort required to organize an international symposium of this quality.

Minor blemishes, which could easily have been avoided, are the rather large number of errors in the references, particularly in the titles of papers in foreign languages, and the failure to correct the cross-references from one paper in the symposium to another.

There is no internationally accepted nomenclature for the ichthyoses, and it is therefore not surprising that different contributors use different terms and classifications. Dr Marks would have placed dermatologists still further in his debt had he and his international colleagues agreed on nomenclature and on a working classification.

ARTHUR ROOK

Chronobiology in Allergy and Immunology. Edited by J.P. MCGOVERN, M.H. SMOLENSKY and A. REINBERG (1977) Springfield, Illinois: C. C. Thomas. Pp. 283. Price \$21.50

Man has known since time immemorial that certain biological responses are not constant, and whether the desired effect is achieved depends not only how and where the stimuli are applied, but when. This book points out that timing may be equally important in more recondite areas of biology such as the measurement of IgA levels in nasal secretions.

Chronobiology is the study of predictable biorhythms with an innate periodicity. In this book, the editors have incorporated some of their own studies with the proceedings of a workshop held in 1973 at the 8th International Conference of Allergology, with the stated aim of promoting the application of chronobiology to clinical practice and research in allergy. The subjects covered include the methodology of chronobiology, and the rhythmicity of allergic responses, including temporal variations in airway patency and cutaneous sensitivity to histamine. There is a useful chapter on the relationship between circadian rhythms and steroid therapy, followed by sections on circadian variations in lymphocyte traffic, serum protein fractions and immune responses in rodents.

This is clearly an important subject, and the book provides several examples which show how chronobiological studies have improved both our understanding of allergic phenomena, and the management of individual patients. The basic concepts are simple, but unfortunately the book is written in an unnecessarily obscure style which makes heavy reading for the neophyte. Those dermatologists who, for example, wouldn't know a bathyphase from a Bath-bun will need to turn to the glossary, where they will learn that the bathyphase is the acrophase plus $180^\circ \pm 15^\circ$, and the acrophase is a measure of crest time by the curve or other function used to approximate a rhythm. They will encounter the cosinor procedure on page 9, but not until page 38 will they discover that biological rhythms can be approximated to a cosine function, and Halberg's cosinor analysis is useful to find the best fitting cosine function. The diagrams too are complicated, and do little to clarify the text. This book cannot therefore be recommended as an introduction to chronobiology for clinicians and it will probably be mainly used as a reference text by immunology research workers. One fears, however, that it may have already become outdated in this rapidly expanding field, and one is forced reluctantly to agree with Sir Winston Churchill's verdict on the name of Mr Bossom, that it is neither one thing nor the other.

J.L. BURTON

Progress in Allergy, vol. 24. Edited by P. KALLOS, B.H. WAKSMAN and A.L. DE WECK (1978) Basel: Karger. Pp. 366. XIV. 29 fig. 5 tab. Price SFr./DM 149.

This series has now established itself as contributing reviews of major importance in immunology in all its ramifications. Nearly every one of the series has contained something of direct relevance to dermatology, this one even more than most. The pattern of four or five up-to-date reviews by acknowledged experts is maintained. The bibliography is vast, this time with nearly 2000 references.

The first article discusses the techniques for detecting immune complexes in human diseases, emphasizing that immune complexes are a normal way of removing antigens efficiently and by no means always harmful. The review is more concerned with the pros and cons of the various techniques than with an assessment of immune complexes in individual diseases. There follows a very long paper on schistosomiasis, from which 8% of the world's population is said to suffer. This might be expected to impinge on dermatology in but a limited way (the histology of sites of cercarial penetration is discussed in some detail) but the article discusses general principles of defence against parasites, the good and harmful effect of the various types of immune reaction, as well as a good miniature review of the eosinophil.

Next comes a paper on granulomatous infiltration, both of the foreign body and of the hypersensitivity type, a constructive way of dealing with certain types of pathogen, even though inappropriate with foreign chemicals.

Silberberg-Sinakin, Baer and Thorbecke contribute an excellent review on the Langerhans cell, updating the reviews of some 5 years ago. They emphasize that these cells, although found most obviously in the epidermis, are circulating cells and a potential link with the lymphoid tissues. The possible role in and damage by immunological reactions of various types are reviewed. Not all the answers are available, but we have come a long way from the effete melanocyte of the last decade.

Finally comes a most useful review on the mechanisms of histamine release in anaphylactoid and anaphylactic reactions with all its relevance to inflammation, atopic disorders and urticaria.

Yet again the editors must be congratulated on assembling their team and on achieving reviews with such a good proportion of recent references (53 from 1977 out of 203 in the first paper).

R.H. CHAMPION

Recent Advances in Dermatopharmacology. Edited by PHILLIP FROST, EDWARD C. GOMEZ and NARDO ZAIAS (1978) New York: Spectrum Publications. Pp. 272. Price £14.

'Dermatopharmacology' is not about the pharmacology of the skin; it is about the clinical pharmacology and therapeutic applications of a number of miscellaneous topical and systemic agents which are used or have a potential use in skin disease. It consists of 21 contributions from clinicians, most of whom are dermatologists, and from non-medical scientists including several from the pharmaceutical industry. The stated aim of the book is to focus attention on applications of advanced technology to problems of clinical pharmacology of dermatological preparations, to provide an insight into the present state of knowledge on this subject and to indicate to the reader possible future developments. The impression gained from reading the book is that progress since Paul Ehrlich studied the interactions between organic arsenicals and the skin 60 years ago has been disappointingly slow. Although clinical pharmacology is now a well established scientific discipline, the limiting factor in applying it to the skin is ignorance of the biology of normal skin, and of its aberrations in skin disease.

The contributions themselves are uneven in quality. The first two chapters deal with the chemical pharmacology of 'microparticle' griseofulvin preparations. The authors present evidence that dispersal of griseofulvin in a polyethylene glycol matrix results in crystalline particles of griseofulvin of approximately 0.01-0.1 μm diameter compared with 10 μm for current griseofulvin preparations. They then go on to provide convincing evidence that blood levels achieved by a given dose of conventional griseofulvin can be achieved by half the dose of the 'microparticle' preparation. Since no data are presented to indicate greater consistency of absorption of the new preparation and since there is nothing to suggest that use of

this preparation will achieve greater efficacy or reduced toxicity, the advantage of microparticle griseofulvin over presently available griseofulvin isn't obvious.

C. Kirkpatrick draws attention to the immunological heterogeneity of patients with chronic mucocutaneous candidiasis. Transfer factor by itself was clinically ineffective, although anergic patients consistently developed positive candida intradermal tests. However, if transfer factor treatment was preceded by clearance of candidiasis by intravenous amphotericin B then sustained remissions resulted, suggesting that, although ineffective by itself, transfer factor's role in this disorder may be to prevent relapses. Controlled trials of this combination are awaited. However, a double blind randomized study was carried out at Stanford in children with multiple warts (perhaps the only double blind study of transfer factor) with negative results.

Uncontrolled observations by physicians form the crudest test of relative activity of different topical steroid preparations. However, R.B. Stoughton points out that sales volumes in the U.S. accurately reflect the ranking of topical steroids by vasoconstriction and other assay methods. Despite the loud protestations of the pharmaceutical industry there is no convincing instance of development of a topical or systemic steroid in which therapeutic efficacy has been successfully dissociated from toxicity. Whether research efforts ought to be directed towards this goal, or whether attempts to develop non-steroid drugs of equivalent potency should be given priority is an important problem with which the author doesn't deal. A study by Gomez and colleagues of adrenal suppression following treatment with potent topical steroids also leaves important questions unanswered, and may lull the unwary reader into a false sense of security. In a double-blind randomized study the authors find that only 2 of 44 psoriatic patients using either of two potent steroids without occlusion showed evidence of adrenal suppression. However, assessment of adrenal function was apparently based solely on plasma cortisol levels, and patients with normal resting cortisol levels may have clinically important abnormalities of pituitary adrenal function. It would have been helpful if the authors had reviewed methodology for assessment of pituitary adrenal function, and then in this context clearly stated what can and cannot be concluded from the tests they had done.

The chapter on topical non-steroid anti-inflammatory agents, potentially of great interest to this reviewer, was disappointing. The author gives data on the inhibiting actions of non-steroid drugs on prostaglandin synthetase obtained from seminal vesicle tissue but doesn't draw attention to the important tissue specificity of this enzyme in respect of sensitivity to drug inhibition. Although the experimental evidence for the suppressive action of indomethacin and related compounds on U.V. erythema is summarized, the underlying assumption that the observed effects are due to inhibition of prostaglandin activity isn't questioned by the author.

The use of gold injections in pemphigus vulgaris is described by P. Frost and E. Gomez. Although initially these workers used gold thiomalate (myocrisin), experience of an anaphylactoid reaction with this agent prompted them to change to gold thioglucose which is well tolerated and just as effective. That 8 out of 18 patients have, after initial control by gold, been able to sustain remissions of 8-28 months without any treatment is impressive. Equally impressive is the high incidence of side effects attributable to gold (7 out of 18, including agranulocytosis (1), erythema nodosum (1) and nephrotic syndrome (1)).

The expected chapter on PUVA treatment for psoriasis from Boston (J.A. Parrish) is a succinct review of the present state of the art. Parrish is unimpressed by the prophets of doom who predict an epidemic of actinic cancer in patients thus treated. His point of view deserves sympathy, but his suggestion that PUVA treatment, because of its tanning effect, protects against solar damage seems slightly euphoric. Chapters on modification of keratinization by lactic acid, pyruvic acid and other hydroxy acids by E. Van Scott and on the neglected subject of topical insect repellants by H. Maibach are worth reading. The chapter by H.P. Baden on hyperkeratosis contains an excellent short and simple review of the molecular biology of keratinization.

For the reader who is prepared to be critical and selective, this book offers much of interest and of potential use to the practising dermatologist.

M.W. GREAVES

News and Notices

Dermatology Seminar at Hawaii: 1979

The Third Annual Dermatology Seminar at Hawaii starts on the evening of 12 February and ends approximately noon 17 February, at The Hotel Intercontinental, Maui, Kehei, Hawaii. The speakers include several guests and the faculty of the above departments. Enrolment is limited and made on a *first come first serve* basis. For information write to The Departments of Dermatology, at University of California Medical School, San Francisco, California 94143, or Cleveland Clinic, Cleveland, Ohio, or Northwestern University Medical School, Chicago, Ill, U.S.A.

The Third Regional Conference of Dermatology, Bali, Indonesia 23-27 November 1978

The Third Regional Conference of Dermatology will be held in Bali, Indonesia, from 23 to 27 November 1978. The official language of the conference will be English. The scientific programme will include mycology, immunology, histopathology, skin biology, leprosy, occupational dermatoses, light dermatoses and bacterial infections. For information: telephone, cable or write, Dr A. Kosasih, 55A, Jl. Moh. Yamin SH, Jakarta, Indonesia. (Telephone 345083).

Coburn Gold Medal

On 2 May 1978, a presentation of the Coburn Gold Medal was made to Dr Maurice Garretts at the Skin Hospital, Manchester, in the presence of Dr Sheila Coburn. The medal, struck in memory of the late Dr James Coburn, is awarded by a panel of dermatologists for meritorious service to dermatology in the Manchester Region.

University of Cambridge

The Annual Introductory Course on the Biology of the Skin will be held in Fitzwilliam College, Cambridge on 5-8 December 1978.

Details may be obtained from The Postgraduate Medical Centre, Level 6, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ.

Irish Association of Dermatologists

The next meeting of this association will be held in the Mater Hospital, Dublin, on Saturday the 7 October 1978. There will be a clinical meeting in the morning and papers in the afternoon.

Further information may be obtained from: Dr R. S. Matthews, Consultant Dermatologist, Department of Dermatology, Route Hospital, Ballymoney, Co. Antrim.

Department of Dermatology, University of Glasgow

The annual course on histopathology of the skin run by this department is continuing and the dates for the 1979 course are 2-6 April.

This course is based on the histological collection gathered by the late Professor John Milne and is designed to be of value to both dermatologists and pathologists. Lecture discussions and projection sessions and individual microscopy sessions are conducted on an informal basis. Numbers are limited to 18 and therefore early application is advisable. Further details can be obtained from the Director of Postgraduate Medical Education, University of Glasgow, Glasgow G12 8QQ.

Advanced Seminar in Dermatology: Cutaneous Microbiology—Laboratory and Clinical Aspects

This annual seminar is intended for Senior Registrars and Consultants in Dermatology. It will include laboratory and clinical aspects of Bacteriology, Mycology, Parasitology and Virology, and will be held from 23 to 27 April 1979 at the Institute of Dermatology and St John's Hospital for Diseases of the Skin. The course fee is £55.

Details can be obtained from The Dean, Institute of Dermatology, 5 Lisle Street, Leicester Square, London WC2H 7BJ. Closing date for applications: 31 December 1978.

Sixth Annual Pediatric Dermatology Seminar

The Sixth Annual Pediatric Dermatology Seminar will take place in the Galapagos Islands, 17–25 February 1979. There will be daily lectures. CME credit will be awarded. Because space is limited on the M/V Buccaneer, early reservations are suggested. The tuition is \$150.

For information please contact Guinter Kahn, M.D., 16800 N.W. 2 Ave, North Miami Beach, Fla. 33169, U.S.A.

Royal College of Physicians of Edinburgh

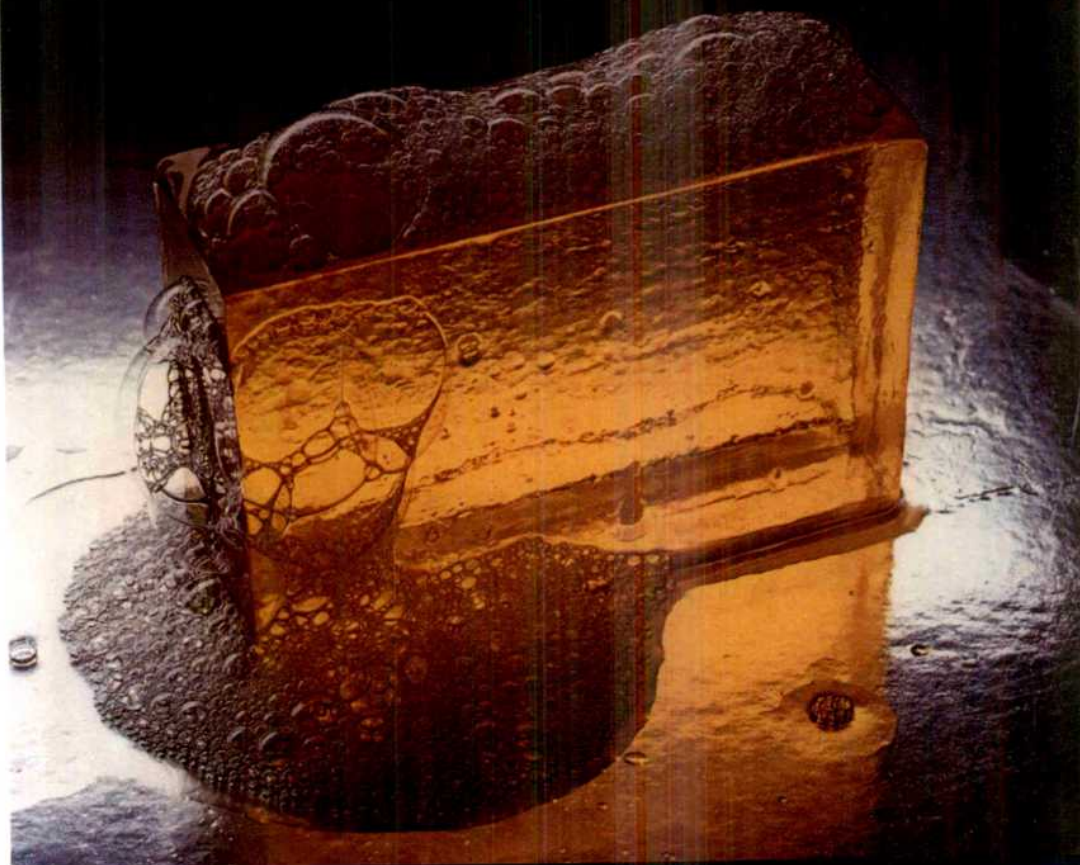
The following dermatologists have recently been elected to the Fellowship of The Royal College of Physicians of Edinburgh:

Dr T.A.J.Dawson; Dr J.A.A.Hunter; Dr K.Keczkes; Dr A.R.Kurwa; Dr R.H.Macdonald; Dr E.L.Smith.

International Symposium on Atopic Dermatitis

An international symposium on atopic dermatitis will be held in Oslo on 7–9 June, 1979. The programme will include epidemiological, clinical, genetic, pathophysiological, immunological and therapeutic aspects of the disease covered by invited lecturers and short communications (10–15 minutes). Ladies programme and possibly workshops and poster session will be arranged. The fee is 300 Norwegian crowns (about £55) for active participants and 200 for family members. Those interested to contribute or attend are invited to correspond with the chairman of the symposium, Professor Georg Rajka (Dept. of Dermatology, Rikshospitalet, Oslo, Norway) before 1 November 1978 and will obtain application forms for presentations and room reservation.

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Workshops:

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2. Principles of Hair Colouring
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Compiled by
Suzanne Alexander and Alan B. Shrank

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1978. 112 pages. Paper, £2.00

Published for the
British Association of Dermatologists by

Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

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1978. 124 pages. Paper, £3.75 net

Blackwell Scientific Publications

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm ³	micrometer(s)	μ m
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	¹³¹ I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on faint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 99, Number 4, October 1978

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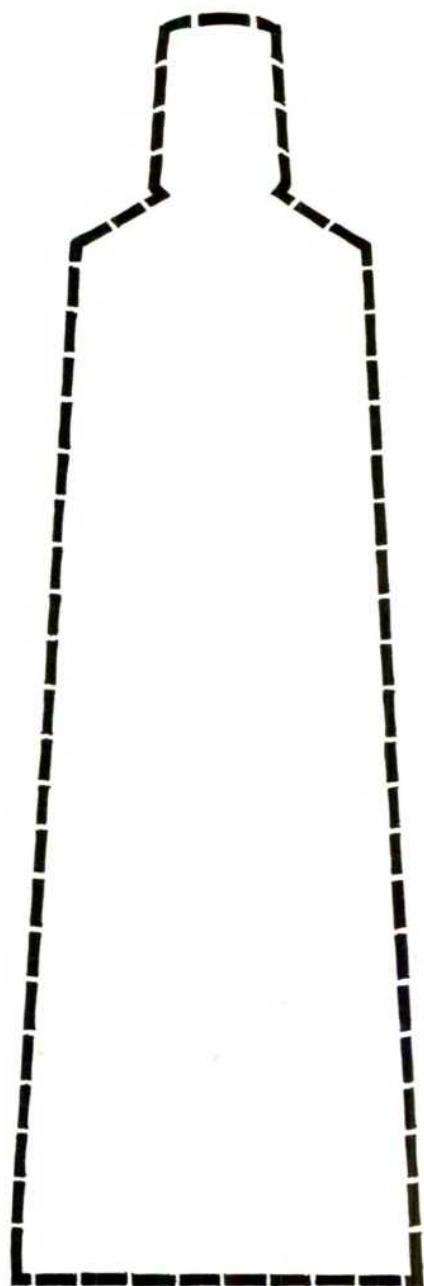
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4/0266

4/0265

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Textbook of Dermatology

Edited by A. Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and F.J.G. Ebling D.Sc. Ph.D. Third Edition, Spring 1979. 2400 pages, 1100 illustrations. Two volumes, about £80.00

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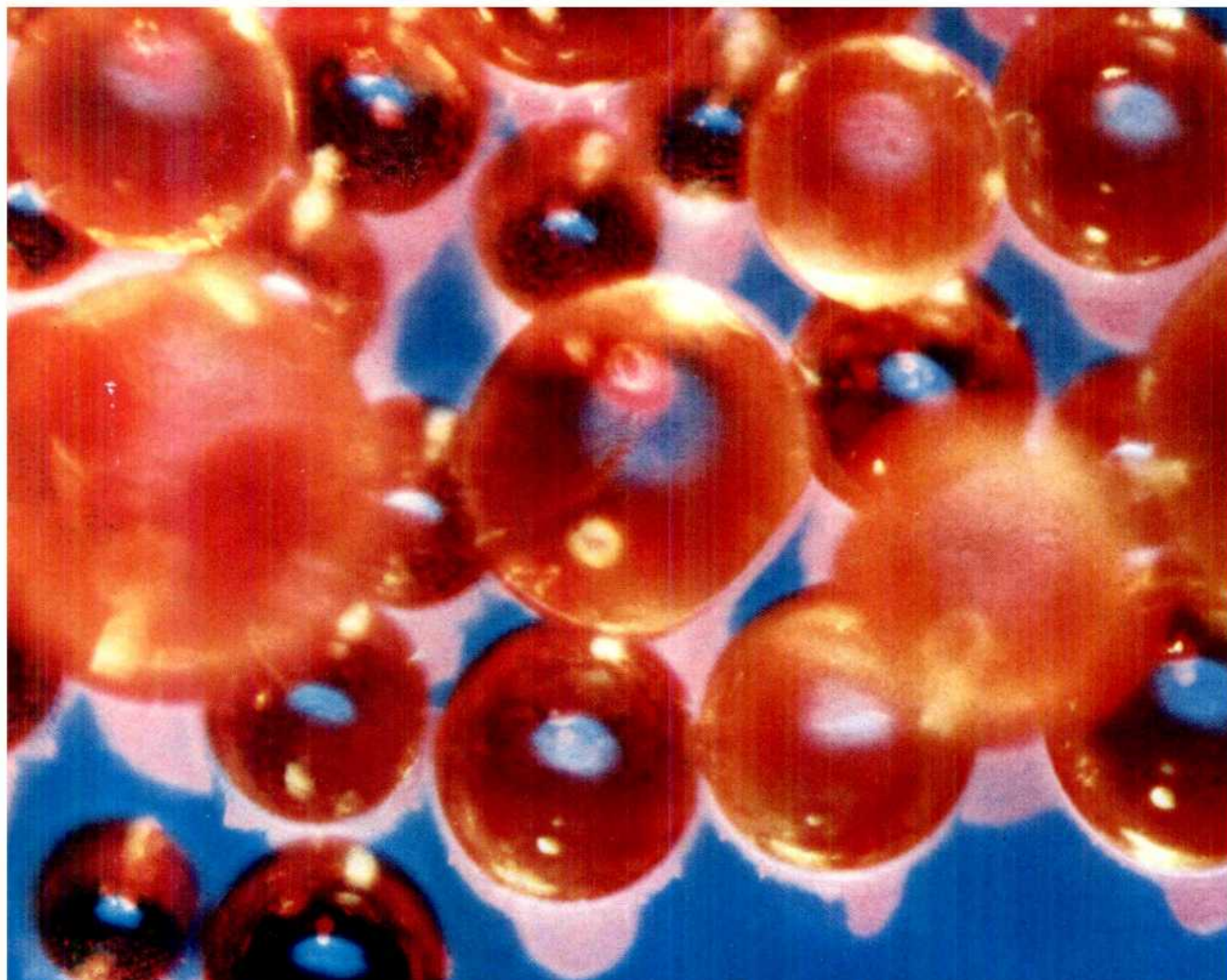
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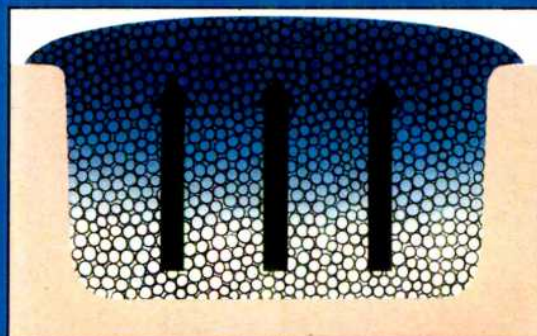
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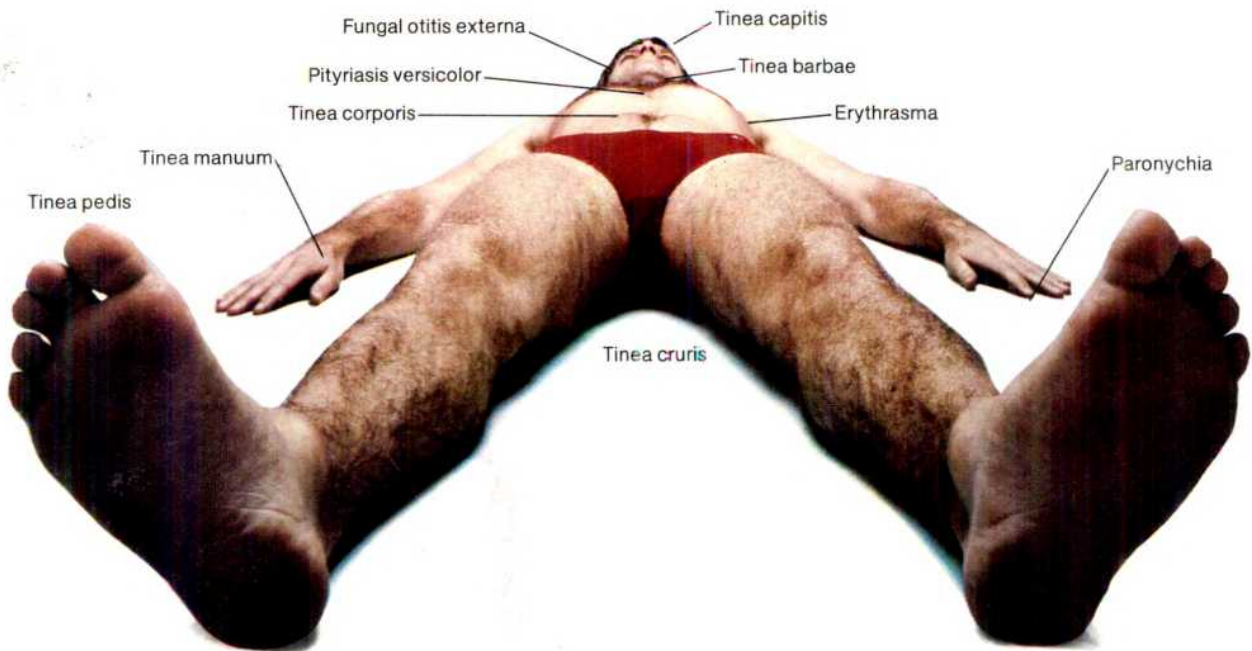
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Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

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(also available in 100 gram tubes)

Presentation Product Licence number

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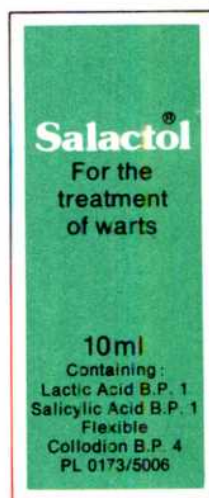
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Brit J Derm. (1971), 84, 366.

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Psoriasis and occlusive vascular disease

CHARLES J. McDONALD AND PAUL CALABRESI

Section of Medicine, Division of Biology and Medicine, Brown University, Department of Medicine, Roger Williams General Hospital, 825 Chalkstone Avenue, Providence, RI 02908, U.S.A.

Accepted for publication 17 April 1978

SUMMARY

To test the hypothesis that psoriasis is associated with an increased incidence of occlusive vascular disease (thrombophlebitis, myocardial infarction, pulmonary embolization, and cerebrovascular accident), the clinical records of 323 psoriatic and 325 non-psoriatic patients admitted to the dermatology service of the Roger Williams General Hospital were examined. The data obtained in this study suggest that (1) the occurrence rate of occlusive vascular disease is significantly greater in the psoriatic than in the non-psoriatic dermatological patient. This is particularly true in the male population; (2) psoriasis predisposes to occlusive vascular disease; and (3) the psoriatic patient with certain predisposing factors is at greater risk of experiencing an occlusive vascular episode than both the non-predisposed psoriatic and the non-psoriatic dermatological patient.

It is well known among dermatologists and dermatopathologists that patients with psoriasis have microvascular abnormalities in normal skin as well as in the skin affected with psoriasis (Braverman, Cohen & O'Keefe, 1972; Braverman, 1972; Levi & Curri, 1973). However, no one has previously associated psoriasis with an increased incidence of large vessel disease. Reed *et al.* (1973) proposed an association between psoriatic arthritis and occlusive vascular disease. Our present interest in this area was stimulated by a number of uncontrolled observations that appeared to associate the administration of a specific antimetabolic agent to patients having severe, recalcitrant psoriasis and an increased occurrence of occlusive vascular episodes in these patients. These observations led us to search the current literature for reports of psoriasis and occlusive vascular disease occurring in both treated and untreated patients and to a rapid review of a representative sample of the clinical records of our untreated psoriatic patients. Both reviews indicated that psoriatic patients experienced more episodes of occlusive vascular disease than would be expected in a normal population (Reed *et al.*, 1961; Nyfors, 1968; Black *et al.*, 1964; Roenigk, Fowler-Bergfeld & Curtis, 1969; Ryan & Baker, 1969). These findings led us to hypothesize that there is a greater than normal incidence of occlusive vascular disease associated with psoriasis (McDonald & Calabresi, 1973a,b,c).

Presented at the 58th Annual Session of the American College of Physicians April 1977.

Reprint requests to: Dr Charles J. McDonald, Department of Medicine, Roger Williams General Hospital, 825 Chalkstone Avenue, Providence, RI 02908, U.S.A.

0007-0963/78/1100-0469 \$02.00 © 1978 British Association of Dermatologists

We then reviewed the clinical records of all psoriatic patients and a similar number of non-psoriatic dermatological patients seen and examined by one of us (C.J.M.) at the Roger Williams General Hospital from 1 July 1968 to 31 December 1972. We now wish to report the findings of a retrospective study designed to test the hypothesis that the psoriatic patient suffers an abnormally high incidence of, and has a greater predisposition to, occlusive vascular disease (i.e. coronary thrombosis, thrombophlebitis, cerebrovascular accidents, and pulmonary embolization).

MATERIALS AND METHODS

A total of 648 patients' records from the Roger Williams General Hospital were sequentially selected and examined using information entered in each record from 1 July 1968 to 31 December 1972. The study was limited to the number of occlusive vascular episodes which were recorded as occurring between 1 January 1968 and 31 December 1972. All occlusive vascular episodes had been previously documented by appropriate clinical and laboratory examinations. Typical diseases of the non-psoriatic patient group included atopic eczema, eczematous dermatitis, acute and chronic allergic contact dermatitis, urticarias (acute and chronic), collagen vascular diseases, alopecias, ichthyoses, etc.

The data retrieved from the records consisted of the following : (a) sex, (b) age, (c) age at the onset of psoriasis, (d) percent of body coverage of psoriasis, (e) history of predisposing factors other than psoriasis, (f) the type of occlusive vascular incident (repeated episodes of the same disease were totalled and counted as a single occurrence. Occurrences of dissimilar diseases in the same patient were identified separately and recorded as such), and (g) age at the time of the occlusive vascular event.

Data retrieval for non-psoriatic patients followed the same format except for items (c) and (d).

Each psoriatic patient was matched with a non-psoriatic patient of similar age (within 5 years) and having a recorded general medical history of similar completeness. The study population contained approximately equal numbers of males and females. Females receiving birth control pills were not admitted to the study.

Several analytical approaches were employed in examining the hypothesis: (a) the incidence of occlusive vascular disease in the psoriatic patient was contrasted with that of the non-psoriatic patient population, (b) the predisposition of the psoriatic population to occlusive vascular disease was contrasted with that of the non-psoriatic population, (c) the psoriatic population was examined alone to see if a relationship existed between the extent and duration of psoriasis and the likelihood of occlusive vascular disease. Additional information obtained for analysis included: (a) association between age, sex, predisposition, and the incidence of occlusive vascular disease, (b) relative frequency of different occlusive vascular episodes, (c) relative significance of psoriasis in the event that it proved to be a predisposition towards occlusive vascular disease.

To stratify the populations into predisposed and non-predisposed groups, a list of factors predisposing and possibly predisposing to occlusive vascular episodes was derived from the literature (Sartwell, 1969). Predisposing factors included a past or present history of heart disease (angina, valvular heart disease, cardiac arrhythmias, arteriosclerotic heart disease, congestive heart failure, etc.), hypertension, deep vein varicosities, chronic venous stasis and peripheral oedema, obesity, anaemia, diabetes mellitus, extensive abdominal surgery, cirrhosis of the liver with peripheral oedema, previously elevated serum clotting factors such as elevated fibrinogen levels, and abnormal lipid profiles. Possible predisposing factors included family history of diabetes mellitus, previous limited abdominal surgery, liver disease, rheumatic heart disease with and without valvular defects, family history of clotting episodes, and family history of cardiovascular disease.

An analysis of the separate categories of occlusive vascular disease was not performed because the number of positive responses within each category was small.

RESULTS

Table 1 shows the occurrence rate of venous and arterial occlusive vascular disease in predisposed and non-predisposed psoriatic and non-psoriatic males and females above and below 50 years of age. In each age/sex group rates of occurrence of occlusive vascular disease were significantly higher ($P < 0.05$)

TABLE 1. Occlusive vascular disease occurrence rate per patient group (648 patients)*

Sex and age group (years)	Predisposition	Type of occurrence					
		Arterial		Venous		Combined (arterial and venous)	
		Psoriatic	Non-psoriatic	Psoriatic	Non-psoriatic	Psoriatic	Non-psoriatic
Male Age < 50	Predisposed	3/8†	1/10 (3.75)	0/8	0/10 (0)	3/8	1/10 (3.75)
	Non-predisposed	37.5‡	10.0	0.0	0.0	37.5	10.0
Male, Age ≥ 50	Predisposed	1/61	0/60 (0.98)	1/61	0/60 (0.98)	2/61	0/60 (1.97)
	Non-predisposed	1.6	0.0	1.6	0.0	3.3	0.0
Female, Age < 50	Predisposed	12/30	3/24 (9.6)	1/30	4/24 (0.8)	13/30	7/24 (10.4)
	Non-predisposed	40.0	12.5	3.3	16.7	43.3	29.2
Female, Age ≥ 50	Predisposed	0/42	0/37 (0)	0/42	0/37 (0)	0/42	0/37 (0)
	Non-predisposed	0.0	0.0	0.0	0.0	0.0	0.0
Male, Age < 50	Predisposed	1/11	1/23 (2.09)	4/11	2/23 (8.36)	5/11	3/23 (10.5)
	Non-predisposed	9.1	4.3	36.4	8.7	45.4	13.0
Female, Age ≥ 50	Predisposed	0/71	0/77 (0)	0/71	0/77 (0)	0/71	0/77 (0)
	Non-predisposed	0.0	0.0	0.0	0.0	0.0	0.0
Male, Age < 50	Predisposed	9/49	4/40 (7.35)	6/49	3/40 (4.90)	15/49	7/40 (12.2)
	Non-predisposed	18.4	10.0	12.2	7.5	30.6	17.5
Female, Age ≥ 50	Predisposed	0/51	0/54 (0)	0/51	0/54 (0)	0/51	0/54 (0)
	Non-predisposed	0.0	0.0	0.0	0.0	0.0	0.0

* P-values (a) Psoriasis vs non-psoriasis $P < 0.005$

(b) Predisposed psoriasis vs predisposed non-psor. $P < 0.005$

(c) Predisposed psoriasis vs non-predisposed psor. $P < 0.0005$

(d) Predisposed psoriasis vs non-predisposed non-psor. $P < 0.0005$

(e) Non-predisposed psoriasis vs predisposed non-psor. $P < 0.0005$

† 3/8 = 3 out of 8 patients

‡ 37.5 = 37.5%

Numbers in parentheses would be expected number of occurrences if psoriatics' occurrence rates applied to non-psoriatics.

in predisposed psoriatic patients than in predisposed non-psoriatic patients. Virtually no occlusive vascular episodes occurred in non-predisposed patients, whether psoriatic or not. In predisposed psoriatic males arterial episodes predominated. In predisposed psoriatic females below 50 years of age venous disease predominated, whereas arterial disease predominated in females over 50.

Additionally, Table 1 shows that there is a significant difference in the number of occlusive vascular episodes experienced by non-psoriatic patients versus the number of occurrences that would have been expected among them if they had experienced the same disease rate as psoriatics. For example, if the

predisposed non-psoriatic group of 10 male patients below age 50 had experienced the same rate of occurrence of arterial disease as was observed in the same age group of predisposed psoriatics, they would have suffered approximately 3.75 clotting episodes instead of a single episode.

TABLE 2. Observed vs expected occlusive vascular occurrences (325 non-psoriatics)

	Observed	Expected	Observed rate (%)	Expected rate (%)	Relative risk
Arterial	9	23.8	2.8	7.3	2.6
Venous	9	15.0	2.8	4.6	1.6
Combined	18	38.8	5.5	11.9	2.2

Table 2 summarizes the observed versus expected occurrence of arterial and venous disease among the combined group of 325 non-psoriatic patients (i.e. the amount of vascular disease actually observed in the group of non-psoriatic patients versus what they would have been expected to have experienced if vascular disease was as prevalent in the group as it was in the psoriatic population).

Once the significance of various factors was demonstrated, the relationship between occurrence of thrombosis and age and percent body coverage of the psoriatic patients was estimated. Contour graphs in Figs 1 and 2 are helpful in understanding the relationships between the variables of age, duration,

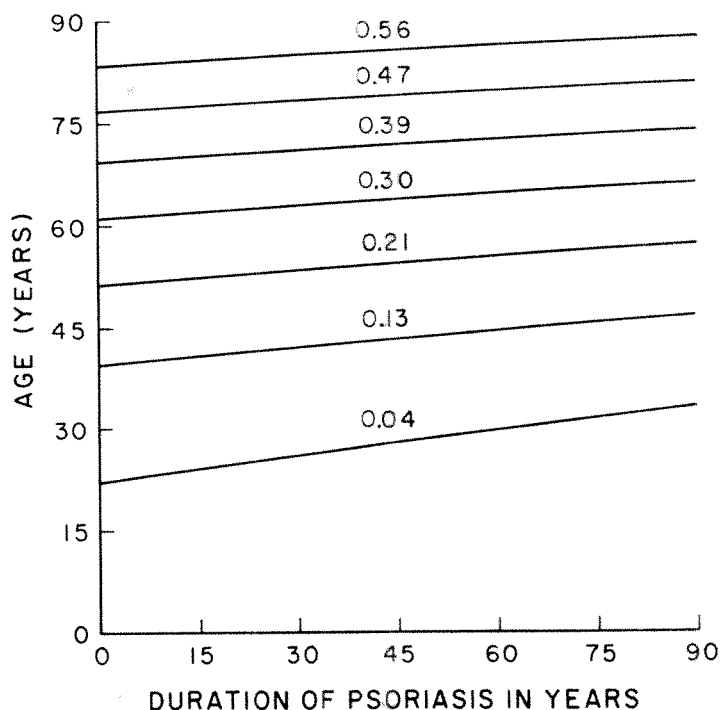


FIGURE 1. Relationship between age and duration of disease and the occurrence of occlusive vascular disease in 323 psoriatic patients.

percent body coverage, and occlusive vascular disease. Duration of psoriatic disease did not appear to have an effect on the patients' likelihood of experiencing an occlusive vascular episode. The percent of the body covered by psoriasis, while not appearing to be as significant as age in effecting an increase in occlusive vascular occurrences, does appear to have some effect, especially in the older age group.

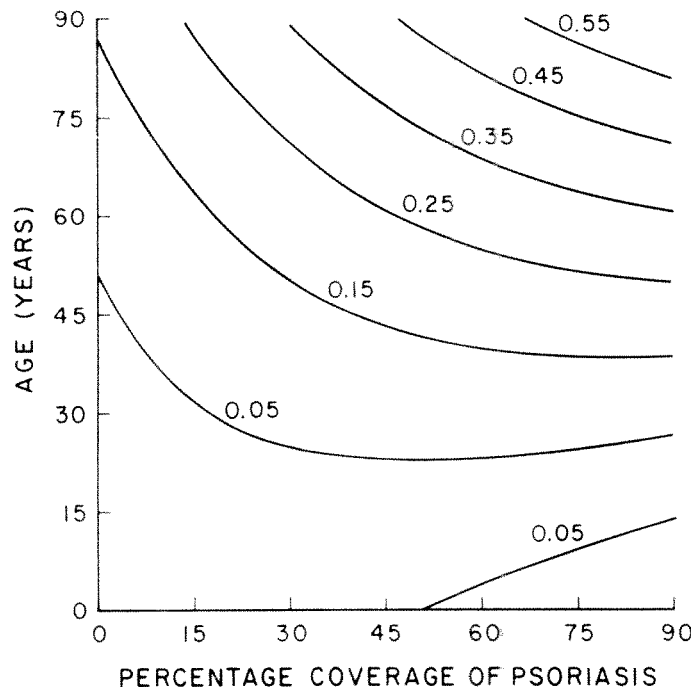


FIGURE 2. Relationship between age and percent body coverage of psoriasis and the occurrence of occlusive vascular disease in 323 psoriatic patients. In order to determine this relationship in any age group and any degree of body coverage using this graph, a straight line is drawn perpendicular to the axis of the age and percent body coverage selected. These two lines will intersect at some point on the graph. This point of intersection will correspond to the expected occurrence rate of occlusive vascular disease.

Example. A 65-year-old male with 15% body coverage has a 15% chance of having an occlusive episode. With 35% body coverage his chances rise to 25%.

DISCUSSION

Using retrospective data, we have attempted to study the hypothesis that patients who have psoriasis suffer an abnormally high prevalence of various occlusive vascular diseases. We recognize the many criticisms of retrospectively gathered data in supporting or refuting a hypothesis (Feinstein, 1973). However, there is broad support for the use of retrospectively gathered data, and it is our belief that such data can, and in this situation do, indicate that our original conjecture is valid and worthy of rigorous testing in a well-planned and properly designed prospective study (Sartwell, 1974).

The data obtained in this study suggest (a) the occurrence rate of occlusive vascular episodes is significantly greater in the psoriatic than in the non-psoriatic patient. In averaging data from the five

age/sex/predisposition categories in which at least one arterial occlusive vascular incident took place, the association between the presence of psoriasis and the occurrence of occlusive vascular disease is statistically highly significant ($P < 0.005$). There appear to be no statistically significant differences among each of the five age/sex/predisposition cells with respect to the degree with which psoriasis and arterial occlusive vascular disease are associated. (b) The predisposed patient not only experienced virtually all of the occlusive vascular episodes reported in this study, but is also at greater risk of developing occlusive vascular disease than the non-predisposed. (c) Females tend to have less arterial but more venous occlusive vascular disease than males. (d) The type and distribution of predispositions in psoriatic patients did not differ to any great degree from those in non-psoriatics. (e) In general, age does not appear to have a cumulative effect on the total incidence of occlusive vascular disease. However, age does appear to produce a greater effect on arterial than on venous disease. (f) Although the evidence is not conclusive, it appears that percent body coverage seems to have an effect on older psoriatic patients' incidence rates. (g) Psoriasis in itself may be a predisposition to occlusive vascular disease.

Why are patients with psoriasis more prone to develop occlusive vascular disease than other dermatological patients? Is it because these patients suffer from an abnormally large number of other diseases which make them more susceptible to occlusive vascular disease? Are there hereditary factors involved? Psoriasis has been demonstrated to be a hereditary disease that can be modified by a number of internal as well as environmental factors. Are psoriatics more prone to develop occlusive vascular disease because there are yet to be described abnormalities in the entire cardiovascular system of the psoriatic patient? Braverman *et al.* (1972), Braverman (1972) and Levi (1973) have described microscopic abnormalities in the cutaneous blood vessels of psoriatic patients. These abnormal blood vessels tend to revert to normal with appropriate antipsoriatic therapy. Many other questions may be asked; yet at present there are no definitive answers.

Although confirmation of the hypothesis that psoriasis is associated with an increased predisposition to and an increased incidence of occlusive vascular disease awaits further testing in controlled prospective studies, we feel that the data presented here add immensely to the unfolding evidence that psoriasis is a generalized metabolic disease with multisystem involvement. The association of psoriasis and joint disease is well known (Baker, Golding & Thompson, 1963; Wright, 1971). Reed *et al.* (1961) described postmortem findings and causes of death in 16 of 86 patients with psoriasis and psoriatic arthritis. Five of the 16 patients died of myocardial infarction (pulmonary embolization was listed as a contributing cause of death in one of the five), and one died of pulmonary haemorrhage and infarction. Reed *et al.* (1961) postulated the association of psoriasis, arthritis, and vascular disease in this manner—'The acute exudative nature of psoriasis may be merely a reflection of severe inflammation seen elsewhere in synovial membranes of the joints, iris, urethra, and the collagen-elastic tissues of the heart and aorta.' Two recently published reports indicate that we should probably rethink the association of psoriasis, methotrexate, and liver disease (Reese *et al.*, 1974; Shapiro *et al.*, 1974). Both studies show that on biopsy the livers of patients with severe psoriasis, whether treated with antimetabolic agents or untreated, demonstrate significant non-specific liver damage—damage that, like arthritis and vascular disease, may very well be associated with a 'psoriasis and systemic disease syndrome.'

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REFERENCES

- BAKER, H., GOLDING, D.N. & THOMPSON, M. (1963) Psoriasis and arthritis. *Annals of Internal Medicine*, **58**, 909.
- BLACK, R.L., O'BRIEN, W.M., VANSOOTT, E.J., AUERBACH, R., EISEN, A.Z. & BUNIM, J.J. (1964) Methotrexate therapy in psoriatic arthritis. *Journal of the American Medical Association*, **189**, 141.
- BRAVERMAN, I.M., COHEN, I. & O'KEEFE, E. (1972) Metabolic and ultrastructural studies in a patient with pustular psoriasis (von Zumbusch). *Archives of Dermatology*, **105**, 189.
- BRAVERMAN, I.M. (1972) Electron microscopic studies of the microcirculation in psoriasis. *Journal of Investigative Dermatology*, **59**, 91.
- FEINSTEIN, A.R. (1973) The epidemiologic trohoc, the ablative risk ratio, and 'retrospective' research. *Clinical Pharmacology and Therapeutics*, **14**, 291.
- LEVI, L. & CURRI, S.B. (1973) Pathological changes of arterio-venous anastomoses (AVA) of the fingertips in normal and affected skin of psoriatic patients. *Archiv für Dermatologische Forschung*, **246**, 59.
- MCDONALD, C.J. & CALABRESI, P. (1973) Thromboembolic disorders associated with psoriasis (Letter to Editor). *Archives of Dermatology*, **107**, 918.
- MCDONALD, C.J. & CALABRESI, P. (1973) Complications of psoriasis (Letter to Editor). *Journal of the American Medical Association*, **224**, 629.
- MCDONALD, C.J. & CALABRESI, P. (1973) Occlusive vascular disease in psoriatic patients (Letter to Editor). *New England Journal of Medicine*, **288**, 912.
- NYFORS, A. (1968) Methotrexate in psoriasis (Letter to Editor). *Lancet*, **ii**, 1251.
- REED, W.B., BECKER, S.W., ROHDE, R. & HEISKELL, C.L. (1961) Psoriasis and arthritis. A clinicopathologic study. *Archives of Dermatology*, **83**, 541.
- REESE, L.T., GRISHAM, J.W., AACH, R.D. & EISEN, A.Z. (1974) Effects of methotrexate on the liver in psoriasis. *Journal of Investigative Dermatology*, **62**, 597.
- ROENIGK, H.H., FOWLER-BERGFELD, W. & CURTIS, G.H. (1969) Methotrexate for psoriasis in weekly oral doses. *Archives of Dermatology*, **99**, 86.
- RYAN, T.J. & BAKER, H. (1969) Systemic corticosteroids and folic acid antagonists in the treatment of generalized pustular psoriasis. Evaluation and prognosis based on the study of 104 cases. *British Journal of Dermatology*, **81**, 134.
- SARTWELL, P.E. (1969) Thromboembolism and oral contraceptives. An epidemiologic case-control study. *American Journal of Epidemiology*, **90**, 363.
- SARTWELL, P.E. (1974) Retrospective studies: A review for the clinician. *Annals of Internal Medicine*, **81**, 381.
- SHAPIRO, H.A., TROWBRIDGE, J.O., LEE, J.C. & MAIBACH, H.I. (1974) Liver disease in psoriatics—An effect of methotrexate therapy? *Archives of Dermatology*, **110**, 547.
- WRIGHT, V. (1971) Psoriatic arthritis. *Bulletin on the Rheumatic Diseases*, **21**, 627.

HLA antigens in persistent palmoplantar pustulosis and its relationship to psoriasis

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SUMMARY

HLA typing was performed on 93 patients with persistent palmoplantar pustulosis. The incidence of either HLA B13 or BW17 was not significantly increased as compared to controls. In addition the frequency of BW35 was no different from controls. However, the present results document a significantly increased frequency (38.7%) of B8. Taken together these data are good evidence that palmoplantar pustulosis is a genetically distinct disease entity from psoriasis vulgaris, despite the increased incidence of psoriasis in the condition.

The aetiology of persistent palmoplantar pustulosis (PPP) remains unknown and its relationship to psoriasis debatable. Many dermatologists still refer to the condition as pustular psoriasis of the palms and soles and certainly the proportion of patients with PPP who also have clinical lesions of psoriasis vulgaris is higher than in the normal population. Enfors & Molin (1971) with the largest published series (272 patients) quote an incidence of 6.1% increasing to 24.4% if all possible cases are included. Everall (1957) found 13 of 70 patients (18.6%) also to have psoriasis and Thomsen & Østerbye (1973) 12 of 40 (30%). In a personal series (JMW) of 185 cases of PPP 30 (16%) either had classical lesions of psoriasis elsewhere on the body at the time of diagnosis or developed them subsequently.

The increased association of HLA B13 and BW17 in psoriasis vulgaris is well documented (Russell, Schultes & Kuban, 1972; White *et al.*, 1972; Svejgaard *et al.*, 1974; Woodrow *et al.*, 1975) and provides an obvious tool for further investigation of this problem. Svejgaard *et al.* (1974), investigating 187 patients, included 31 cases of pustular psoriasis in their series of whom 17 were classified as suffering from PPP. They found these 31 cases to show no excess BW17 or B13 in contrast to 156 patients with ordinary psoriasis. Zachariae *et al.* (1977) typed 97 patients with pustular psoriasis including 27 from the preceding series. The 97 comprised 18 patients with generalized pustular psoriasis (GPP); 14 patients with acrodermatitis continua (AC); 30 patients with localized pustular psoriasis of the palms and soles who had lesions of psoriasis elsewhere on the body (LPP) and 35 patients with localized pustular lesions on the palms and soles and no psoriasis elsewhere (PPP). In

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this last group they found an increased incidence of BW35. No increase in B13 or B17 was found except in the group of LPP. These findings suggested that all forms of pustular psoriasis and psoriasis vulgaris may be different aetiological entities although some patients with LPP might be true psoriatics who also happened to have pustular lesions on the palms and soles. Karvonen *et al.* (1975) also demonstrated no significant deviation in the frequencies of B13 and BW17. They recorded an increase in 'W19' and BW35 in 22 patients with PPP without psoriasis and no increase in 14 patients with PPP, who also had psoriasis. All these authors discriminate between cases of PPP associated with psoriasis and those without. In an attempt to clarify the important question of the relationship, if any, between PPP and psoriasis vulgaris we have carried out HLA typing in a large series of clinically well-documented cases.

PATIENTS AND METHODS

Ninety-three patients (including 2 who were related), 20 men and 73 women, with established PPP were studied. Diagnosis was based on the presence of chronic, recurring, sterile pustulation on the palms and/or soles with characteristic cyclical changes in the pustules from yellow to brown, followed by shedding of the dry scale. Only cases showing active pustulation were included. The age of onset, family history of psoriasis or PPP, nail changes, presence of classical psoriatic lesions elsewhere on the body and presence of arthropathy were recorded.

TABLE 1. Frequency of HLA phenotypes in patients with persistent palmo-plantar pustulosis and controls

Antigen (HLA)	Controls			Patients	
	London (n=280)	Aarhus* (n=562)	Helsinki† (n=326)	(n=93)	χ^2
A1	85 (30.4)	189 (33.6)	55 (17)	34 (36.6)	0.97
A2	142 (50.7)	306 (54.4)	179 (55)	47 (50.5)	0.01
A3	66 (23.6)	165 (29.4)	137 (42)	28 (30.1)	1.25
A9	56 (20.0)	89 (15.8)	59 (18)	18 (19.4)	0.00
A10	19 (6.8)	39 (6.9)	33 (10)	9 (9.7)	0.48
A11	34 (12.1)	73 (13.0)	33 (10)	10 (10.8)	0.03
A28	23 (8.2)	45 (8.0)	39 (12)	10 (10.8)	0.29
B5	32 (11.4)	67 (11.9)	42 (13)	3 (3.2)	4.60
B7	75 (26.8)	197 (35.1)	95 (29)	22 (23.7)	0.21
B8	52 (18.6)	129 (23.0)	59 (18)	36 (38.7)	14.61
B12	96 (34.3)	124 (22.1)	49 (15)	32 (34.4)	0.01
B13	18 (6.4)	16 (2.8)	26 (8)	0 (0)	4.96
B14	19 (6.8)	12 (2.1)	—	3 (3.2)	1.02
B18	16 (5.7)	36 (6.4)	16 (5)	1 (1.1)	2.47
B27	20 (7.1)	47 (8.4)	46 (14)	9 (9.7)	0.32
BW15	40 (14.3)	108 (19.2)	85 (26)	10 (10.8)	0.48
BW17	25 (8.9)	46 (8.2)	13 (4)	7 (7.5)	0.04
BW21	8 (2.9)	6 (1.1)	—	2 (2.2)	0.00
BW22	8 (2.9)	16 (2.8)	23 (7)	5 (5.4)	0.67
BW35	35 (12.5)	73 (13.0)	62 (19)	14 (15.1)	0.21
BW40	62 (22.1)	107 (19.0)	49 (15)	11 (11.8)	4.09

* Data from Svejgaard *et al.* (1974).

† Data from Karvonen *et al.* (1975).

χ^2 =chi square (1 d.f.). Figures in parentheses are frequencies (%).

TABLE 2. Clinical data

	All cases	PPP + psoriasis	PPPP - psoriasis
Total no. of patients	93	19	74
Sex: M	20	5	15
F	73	14	59
Mean age at onset PPP	47 years $\pm$ 14.8	48 years $\pm$ 11.0	47 years $\pm$ 15.7
Family history psoriasis	14	7	7
Family history PPP	5	2	3
Sero-negative arthropathy	4	3	1
Nail changes	22	6	16
Past history GPP	1	—	1

HLA typing was carried out in the Tissue Typing Laboratory, Charing Cross Hospital, London using a modified two-stage lymphocytotoxicity technique (Barnes *et al.*, 1974; Keat & Barnes, 1976) for the 21 antigen specificities shown in Table 1. The 1975 W.H.O. nomenclature for HLA has been adopted. Control samples were obtained from a random panel of 280 normal individuals.

STATISTICS

Chi square (χ^2) values for the differences in HLA antigen frequencies in patients and controls were computed using Yates' correction.

RESULTS

Table 1 shows the HLA antigen frequencies in 93 patients with PPP together with χ^2 values for the differences in frequencies between patient and controls. HLA antigen frequencies in 280 control subjects are given, together with, for comparison, their frequencies in normal Danish (Svejgaard *et al.*, 1974) and Finnish (Karvonen *et al.*, 1975) populations.

The frequencies of BW17 (7.5%), BW35 (15.1%) and B27 (9.7%) are not significantly different from their frequencies in controls. B13 did not occur in any of the 93 patients ($P < 0.05$). There was a significant increase in the frequency of B8 (38.7%) compared to controls (18.6%); $\chi^2 = 14.6$ $P < 0.001$ ($P < 0.02$ after correction for the number of specificities tested). The incidence of A1 was no different in patients and controls and, in addition, the incidence of B8 in the controls (18.6%) was not significantly different from that in either the Danish (23.1%) or Finnish (18%) populations.

Clinical data are recorded in Table 2. Nineteen cases showed clinical evidence of psoriasis and data from these cases are separately recorded and compared to that of the 74 cases showing no evidence of psoriasis. Nine of these had developed psoriasis after the onset of PPP, 5 concurrently, 4 before and 1 unknown. It is noteworthy that the accompanying psoriasis was very mild in these cases, only 2 cases actually seeking treatment for it. There is no statistical significance between these two groups apart from the increased incidence of a family history of psoriasis in those patients with clinical psoriasis as might be expected. On clinical examination there was no consistent difference between the morphological patterns and distribution of lesions on the palms and soles between these two sets of patients.

Table 3 shows the frequencies of HLA antigens in these same two groups compared to controls (from Table 1). BW17 is not increased in either group.

TABLE 3. Frequency of HLA phenotypes in patients with persistent palmo-plantar pustulosis, with and without associated manifestations of psoriasis

Antigen (HLA)	PPP (with psoriasis) (n=19)	PPP (without psoriasis) (n=74)	Controls (London) (n=280)
A1	5 (26.3)	29 (39.2)	85 (30.4)
A2	9 (47.4)	38 (51.4)	142 (50.7)
A3	10 (52.6)	18 (24.3)	66 (23.6)
A9	4 (21.1)	14 (18.9)	56 (20.0)
A10	2 (10.5)	7 (9.5)	19 (6.8)
A11	1 (5.3)	9 (12.2)	34 (12.1)
A28	3 (15.8)	7 (9.5)	23 (8.2)
B5	0 (0)	3 (4.1)	32 (11.4)
B7	5 (26.3)	17 (23.0)	75 (26.8)
B8	8 (42.1)	28 (37.8)	52 (18.6)
B12	5 (26.3)	27 (36.5)	96 (34.3)
B13	0 (0)	0 (0)	18 (6.4)
B14	0 (0)	3 (4.1)	19 (6.8)
B18	0 (0)	1 (1.4)	16 (5.7)
B27	3 (15.8)	6 (8.1)	20 (7.1)
BW15	0 (0)	10 (13.5)	40 (14.3)
BW17	2 (10.5)	5 (6.8)	25 (8.9)
BW21	0 (0)	2 (2.7)	8 (2.9)
BW22	0 (0)	5 (6.8)	8 (2.9)
BW35	1 (5.3)	13 (17.6)	35 (12.5)
BW40	1 (5.3)	10 (13.5)	62 (22.1)

DISCUSSION

The previous series of HLA typing in patients with PPP reported by Svegaard *et al.* (1974), Zachariae *et al.* (1977) and Karvonen *et al.* (1975), in which the distributions of HLA B13 and BW17 were normal, suggested that PPP may be a disease entity distinct from psoriasis vulgaris since in this condition it is well documented that these antigens show an increased frequency. The present results lend considerable support to this hypothesis. The association of PPP with HLA BW35 remarked by both Karvonen and Zachariae was not confirmed and the possible association of 'W19' recorded by Karvonen *et al.* (1975), but not by Zachariae *et al.* (1977), could not be substantiated adequately to define specificities of the 'W19' complex due to a lack of typing sera; the frequency of A29, however, was no different from controls. In the present series the differences noted between so-called LPP and PPP were not substantiated by HLA typing.

The increased frequency of HLA B8 which has been reported as being low in ordinary psoriasis (Beckman *et al.*, 1977) is a new finding which raises several questions. This increase was significant and could not be attributed to linkage disequilibrium with HLA A1 whose distribution in these patients was not significantly different from controls. An increased frequency of HLA B8 has been reported in other dermatological conditions, notably dermatitis herpetiformis (Solheim *et al.*, 1976; Seah *et al.*, 1976), and other disorders including juvenile onset diabetes (Cudworth & Woodrow, 1975), thyrotoxi-

cosis (Grumet *et al.*, 1973) and chronic adrenalitis (Platz *et al.*, 1974) in which autoimmune mechanisms may have a role, at least in part, in their pathogenesis. Husby *et al.* (1973) made immunofluorescence studies on 7 cases of PPP and found immunoglobulin deposits in the pustular areas. It seems possible, however, that this was non-specific staining occurring in polymorph exudates. There has been no other evidence to suggest that the condition is auto-immune.

The possible mechanisms involved in the associations of products of the major histocompatibility complex (MHC) with disease in man are currently under intensive investigation (McDevitt & Bodmer, 1974; Munro & Bright, 1976). If the increased association of HLA B8 with PPP can be substantiated it will be of considerable interest to determine whether other gene products of the MHC such as HLA D or Ia determinants (Van Rood *et al.*, 1975; Sachs & Dickler, 1975; Ting *et al.*, 1976) show an even greater association with this condition.

The relationship of psoriasis and PPP has been much discussed in the past. Barber (1930) and Ingram (1930) initially considered the condition to be a localized pustular variant of psoriasis usually associated with clinical psoriasis elsewhere. Andrews (1935) described pustular bacteride, associated with focal infection, as a separate entity but the role of infection was questioned by Everall (1957) and in England, pustular bacteride, if it exists at all, is generally considered to be a rare variant of PPP. Sachs & Scannone (1945) disputed on histological, clinical and therapeutic grounds that the so-called pustular psoriasis of Barber is in fact related to psoriasis. Ashurst (1964), with an excellent historical review, studied 51 cases and having initially excluded those with clinical psoriasis found only one patient to develop it subsequently. He concluded that there was no demonstrable relationship with psoriasis. Hellgren & Mobacken (1971) studied 85 patients with no previous psoriasis and found none to develop it over 5 years; they noted certain important differences between the two conditions. Enfors and Molin (1971), however, with 272 patients and the high incidence of psoriasis already quoted, could only conclude that there is a close correlation between the two conditions. Baker (1977) called the condition palmo-plantar pustular psoriasis considering it a localized form of pustular psoriasis. Svejgaard, Zachariae and Karvonen distinguished between PPP associated with psoriasis and PPP without psoriasis; their work also suggests that generalized pustular psoriasis and psoriasis vulgaris are different aetiological entities.

One may summarize these points of view by posing and attempting to answer three questions:

(1) Are all cases of PPP really a form of psoriasis as implied by calling the condition pustular psoriasis of the palms and soles?—The results of HLA typing suggest that PPP is genetically distinct from psoriasis. The marked resistance to conventional psoriatic therapy is also against this view.

(2) Are those cases in which PPP is clinically associated with psoriasis distinguishable from the rest in any other way?—Morphologically and genetically this study did not find this to be so. Histologically Thormann & Heilesen (1975) studied 47 biopsies of PPP blind and were unable to distinguish between those cases with psoriasis and those without; moreover, in contrast to Karvonen, in this study both groups had some relatives with psoriasis.

(3) Are all cases not associated with psoriasis identical with Andrews' pustular bacteride, as suggested in some American literature (Domonkos, 1971)?—This is not generally accepted in this country.

PPP emerges as a clear-cut clinical entity of unknown aetiology, apparently genetically distinct from psoriasis but in which there is, nevertheless, an increased incidence of mild psoriasis.

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REFERENCES

- ANDREWS, G.C. & MACHACEK, G.F. (1935) Pustular bacterids of the hands and feet. *Archives of Dermatology and Syphilology*, **32**, 837.
- ASHURST, P.J.C. (1964) Relapsing pustular eruptions of the hands and feet. *British Journal of Dermatology*, **76**, 169.
- BAKER, H. (1977) Psoriasis. In: *Dermatology* (Ed. by R. Marks and P.D. Samman), p. 156. W. Heineman Medical Books Ltd., London.
- BARBER, H.W. (1930) Acrodermatitis continua vel perstans (dermatitis repens) and psoriasis pustulosa. *British Journal of Dermatology*, **42**, 500.
- BARNES, R.M.R., PEGRUM, G.D., GORDON, E.M., WILLIAMS, G.B. & DE WARDENER, H.E. (1974) Assessment of human renal transplantation. *Lancet*, **ii**, 1040.
- BECKMAN, L., BERGDAHL, K., CEDERGREN, B. & LIDEN, S. (1977) Genetic markers in psoriasis. *Acta dermatovenereologica*, **57**, 247.
- CUDWORTH, A.G. & WOODROW, J.C. (1975) HL-A system and diabetes. *Diabetes*, **24**, 345.
- DOMONKOS, A.N. (1971) Recalcitrant pustular eruptions of the hands and feet. In: *Andrew's Diseases of the Skin*, 6th edn., p. 284. W.B. Saunders Co., Philadelphia and London.
- ENFORS, W. & MOLIN, L. (1971) Pustulosis palmaris et plantaris. *Acta dermatovenereologica*, **51**, 289.
- EVERALL, J. (1957) Intractable pustular eruptions of the hands and feet. *British Journal of Dermatology*, **69**, 269.
- GRUMET, C., CONISHI, J., PAYNE, R. & CRISS, J.P. (1973) Association of Grave's disease with HL-A8. *Clinical Research*, **21**, 493.
- HELLGREN, L. & MOBACKEN, H. (1971) Pustulosis palmaris et plantaris. *Acta dermatovenereologica*, **51**, 284.
- HUSBY, G., RAJKA, G. & EEG LARSEN, T. (1973) Immunofluorescence studies in pustulosis palmo-plantaris. *Acta dermatovenereologica*, **53**, 123.
- INGRAM, J.T. (1930) Acrodermatitis perstans and its relation to psoriasis. *The British Journal of Dermatology and Syphilis*, **42**, 489.
- KARVONEN, J., TIILIKAINEN, A. & LASSUS, A. (1975) HL-A antigens in patients with persistent palmo-plantar pustulosis and pustular psoriasis. *Annals of Clinical Research*, **7**, 112.
- KEAT, A.C. & BARNES, R.M.R. (1976) HL-A 27 associated arthritis. *Rheumatology and Rehabilitation*, **15**, 87.
- MCDEVITT, H.O. & BODMER, W.F. (1974) HL-A immune response genes and diseases. *Lancet*, **i**, 1269.
- MUNRO, A. & BRIGHT, S. (1976) Products of the major histocompatibility complex and their relationship to the immune response. *Nature*, **264**, 145.
- PLATZ, P., RYDER, L., STAUB-NIELSEN, L., SVEJGAARD, A., THOMPSEN, M., CHRISTY, M. & NERUP, J. (1974) HL-A and idiopathic Addison's disease. *Lancet*, **ii**, 289.
- RUSSELL, T.J., SCHULTES, L.M. & KUBAN, D.J. (1972) Histocompatibility (HL-A) antigens associated with psoriasis. *New England Journal of Medicine*, **287**, 738.
- SACHS, D.H. & DICKLER, H.B. (1975) The possible role of I region determined cell surface molecules in the regulation of immune responses. *Transplantation Reviews*, **23**, 159.
- SACHS, W. & SCANNONE, F. (1945) so-called 'Pustular psoriasis'. *Journal of Investigative Dermatology*, **6**, 349.
- SEAH, P.P., FRY, L., KEARNEY, J.W., CAMPBELL, E., MOWBRAY, J.F., STEWART, J.S. & HOFFBRAND, A.W. (1976) A comparison of histocompatibility antigens in dermatitis herpetiformis and coeliac disease. *British Journal of Dermatology*, **94**, 131.
- SOLHEIM, B.G., EK, J., THUNE, P.O., BAKLIEN, K., BRATLÆ, A., RANKIN, B., THORESEN, A.B. & THORSBY, E. (1976) HLA antigens in dermatitis herpetiformis and coeliac disease. *Tissue Antigens*, **7**, 57.
- SVEJGAARD, A., STAUB-NIELSEN, L., SVEJGAARD, E., KISSMEYER-NIELSEN, F., HJORTSHØJ, A. & ZACHARIAE, H. (1974) HL-A in psoriasis vulgaris and in pustular psoriasis—population and family studies. *British Journal of Dermatology*, **91**, 145.
- THOMSEN, K. & ØSTERBYE, P. (1973) Pustulosis palmaris et plantaris. *British Journal of Dermatology*, **89**, 293.
- THORMANN, J. & HEILESEN, B. (1975) Recalcitrant pustular eruptions of the extremities. *Journal of Cutaneous Pathology*, **2**, 19.
- TING, A., MICKEY, M.R. & TERASAKI, P.I. (1976) B-lymphocyte alloantigens in caucasians. *Journal of Experimental Medicine*, **143**, 981.
- VAN ROOD, J.J., VAN LEEUWEN, A., KEUNING, J.J. & BLASSÉ VAN OUD ALBAS, A. (1975) Serological detection and partial characterization of human MLC determinants with special reference to B-cell specificity. *Tissue Antigens*, **5**, 73.

- WHITE, S.H., NEWCOMER, V.D., MICKEY, M.R. & TERASAKI, P.I. (1972) Disturbance of HL-A antigen frequency in psoriasis. *New England Journal of Medicine*, **287**, 740.
- WOODROW, J.C., DAVE, V.K., USHER, N. & ANDERSON, J. (1975) The HL-A system and psoriasis. *British Journal of Dermatology*, **92**, 427.
- ZACHARIAE, H., OVERGAARD PETERSEN, H., KISSMEYER-NIELSEN, F. & LAMM, L. (1977) HL-A antigens in pustular psoriasis. *Dermatologica*, **154**, 73.

Multinucleate epidermal cells in non-neoplastic dermatoses

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SUMMARY

Multinucleate epidermal cells (MEC) originated from keratinocytes were observed histologically in 92 of 197 cases of non-neoplastic dermatoses, such as lupus erythematosus (26 of 70), lichenoid eruptions (26 of 47), Hailey-Hailey disease (5 of 5), psoriasis vulgaris (20 of 49) and so on. Most of these cells had two nuclei, and a few had three nuclei. MEC usually showed perinuclear bands of eosinophilic material which also showed positive staining for epidermal fibres (tonofilaments). In all cases of Hailey-Hailey disease, close correlation between formation of MEC and dyskeratotic tendency was observed. From these findings, it is concluded that the mechanism of formation of MEC in these dermatoses may be similar to that of Bowen's disease, in which dyskeratotic tonofilaments become entangled with the spindles of the mitotic apparatus so that normal cell division cannot take place. It seems likely that MEC are a manifestation not only of malignant dyskeratosis but also of benign one.

There appear to be only a few reports on multinucleate epidermal cells (MEC) in non-neoplastic and non-viral conditions (Pinkus, 1951; Ofuji & Horio, 1970; Omar *et al.*, 1977; Shahrads & Marks, 1977), although these cells are commonly seen in both viral skin diseases and malignant or pre-malignant epidermal tumours. The mechanism of formation of MEC in these conditions remains obscure. In this study, MEC were not infrequently encountered in a large number of non-neoplastic and non-viral dermatoses, and the histopathogenesis of multinucleation of epidermal cells is discussed.

MATERIALS AND METHODS

One hundred and ninety seven cases of non-neoplastic dermatoses (Table 1) seen at the Department of Dermatology, Keio University School of Medicine from 1973 to 1977 were examined.

Formalin-fixed and paraffin-embedded sections were stained with haematoxylin and eosin. Eight cases which showed typical MEC were also examined by periodic acid-Schiff (PAS) stain with and without diastase digestion, Patzelt stain (Patzelt, 1926) and Unna-Pappenheim stain.

In haematoxylin and eosin-stained sections, liquefaction degeneration of the basal cells, a dyskeratotic

tendency, especially individual cell keratinization, frequency of mitosis, epidermal thickness, dermal cellular and actinic elastosis as well as MEC were examined.

RESULTS

Rates of appearance of MEC in each disease or group of diseases (Table 1) were relatively high (mean

TABLE 1. One hundred and ninety-seven cases of dermatoses examined in this study

Dermatoses	No. of cases	No. of cases with binucleate cells	No. of cases with trinucleate cells
Lupus erythematosus (systemic and discoid)	70	26	3
Lichenoid eruptions (Lichen planus and lichenoid drug eruptions)	47	26	2
Erythema multiforme	6	4	0
Sweet's disease	5	3	0
Hailey-Hailey disease	5	5	0
Psoriasis vulgaris	49	20	1
Livedo group	15	8	1
Total	197	92	7

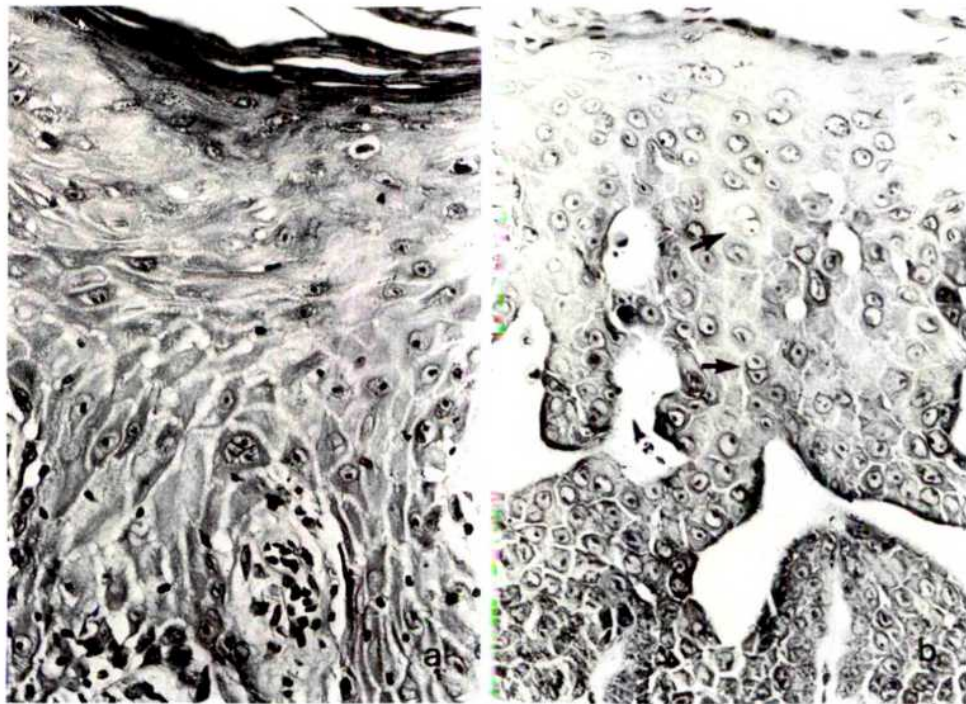


FIGURE 1. (a) Lichenoid drug eruption to show a trinucleate cell in the suprabasal area of the epidermis. (b) Hailey-Hailey disease to show binucleate cells (arrows) in the prickle cell layer of the epidermis (H & E, $\times 350$).

47%); the highest was 100% in Hailey-Hailey disease and the least was about 40% in psoriasis vulgaris. MEC showed intercellular bridges between neighbouring keratinocytes so that they were likely to be originated from keratinocytes. MEC consisted largely of binucleate cells (Fig. 1a). Trinucleate cells (Fig. 1b) were found in only 7 cases. Cells with more than three nuclei were not found in these cases. Few nuclei of MEC differed from those of mononuclear epidermal cells. MEC were distributed in the different cell layers of the epidermis, from the basal layer to the granular layer, and usually showed perinuclear bands of eosinophilic material in haematoxylin and eosin stain (Fig. 1a and b).

MEC contained various amounts of diastase-digestive PAS positive substance (glycogen), and an average amount of this substance did not exceed that of mononuclear epidermal cells. By Patzelt stain MEC usually showed dense perinuclear bands of epidermal fibres (tonofilaments) (Fig. 2a and b). Unna-Pappenheim stain for nucleic acids revealed no difference between MEC and mononuclear epidermal cells.

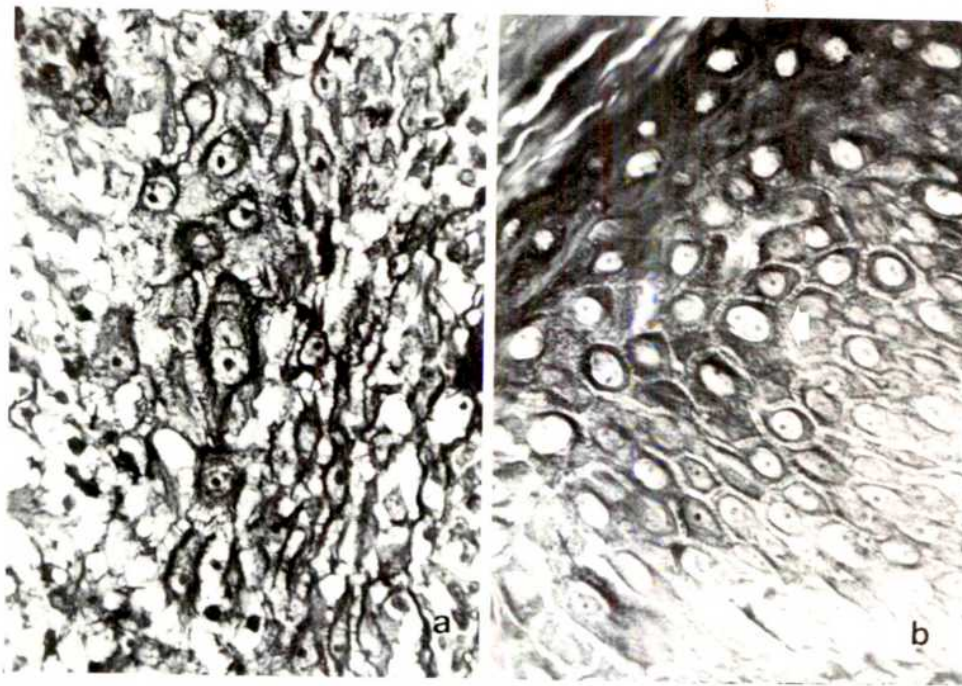


FIGURE 2. (a) Lichenoid drug eruption showing a binucleate cell with perinuclear bands of epidermal fibres. (b) Lichen planus showing a binucleate cell (arrow) with dense perinuclear bands of epidermal fibres (Patzelt stain, $\times 700$).

Relationships between formation of MEC and other histological changes, such as liquefaction degeneration of the basal cells, dyskeratotic tendency, frequency of mitosis, epidermal thickness, and cellular infiltrates and actinic elastosis in the upper dermis, were variable (Tables 2-4). Close correlation was found between formation of MEC and dyskeratotic tendency expressed by individual cell keratinization (Table 3), especially in Hailey-Hailey disease. Some interrelation was found between formation of MEC and epidermal thickness and cellular infiltrates in lupus erythematosus. No correlation was observed between formation of MEC and the other histological changes (Tables 2 and 4).

TABLE 2. Correlation between formation of MEC and liquefaction degeneration of the basal cells

Dermatoses	Liquefaction degeneration	Rate of appearance of MEC
Lupus erythematosus and lichenoid eruptions	+	52/117 (44%)
Psoriasis vulgaris	—	20/49 (41%)

TABLE 3. Correlation between formation of MEC and dyskeratotic tendency (individual cell keratinization)

Dermatoses	Rate of appearance of individual cell keratinization	No. of cases with both MEC and individual cell keratinization
Hailey-Hailey disease	5/5	5
Lichenoid eruptions	29/47	16
Lupus erythematosus	29/70	16
Psoriasis vulgaris	5/49	1

TABLE 4. Correlation between formation of MEC and frequency of mitosis

Dermatoses	No. of cases with mitotic figures	No. of cases with both MEC and mitotic figures
Lupus erythematosus	3	1
Lichenoid eruptions	6	5
Psoriasis vulgaris	18	6

COMMENT

In 1951, Pinkus observed a fair number of binucleate cells in the epidermis at 24 h after stripping of the horny layer and stated that direct nuclear division (amitosis) might have occurred. Ofuji & Horio (1970) found multinucleate epidermal cells within or near the spongiotic areas of guttate parapsoriasis and eczematous dermatitis, and considered that amitosis in the degenerative process of affected epidermal cells might play a role in the formation of MEC. Recently Shahrads & Marks (1977) observed MEC in the epidermis of the lesions of erythema ab igne and interpreted this as epidermal atypia which might indicate malignant potentiality. Omar *et al.* (1977) found MEC in the epidermis of the uninvolved skin of psoriatic patients having PUVA therapy and considered that amitotic nuclear division might take place. No direct evidence of amitosis and malignant transformation, however, has been found in the above studies or in this study.

On the other hand, in Bowen's disease, which is one of the neoplastic dermatoses with prominent MEC, two kinds of mechanism of formation of MEC have been proposed after ultrastructural studies (Olson *et al.*, 1969; Seiji & Mizuno, 1969; Lever & Schaumburg-Lever, 1975); in the first, an entire dyskeratotic cell has been 'cannibalized' by another keratinocyte and is located within the cytoplasm of the phagocytosing cell; and in the second, multiple nuclei lie in the centre of the cell surrounded by dyskeratotic tonofilaments. In the second mechanism it seems that the dyskeratotic tonofilaments become entangled with the spindles of the mitotic apparatus and interfere with the normal cell division, and that cellular division cannot occur even though nuclear division can take place.

In this study it has been shown that MEC usually have perinuclear bands of eosinophilic material which has been proved to be epidermal fibres (tonofilaments) by Patzelt stain. This finding suggests that in non-neoplastic and non-viral dermatoses MEC may be produced by a process similar to that in Bowen's disease, especially the second one. In other words, dyskeratotic change might play a role in the formation of MEC not only in Bowen's disease but also in the dermatoses examined in this study. This hypothesis may be supported by close correlation between dyskeratotic tendency and the formation of MEC in Hailey-Hailey disease. Furthermore, this cannot be denied by the finding that MEC were formed in the epidermis with PUVA therapy (Omar *et al.*, 1977), since ultraviolet light-induced individual cell keratinization has already been demonstrated by electron microscopy (Olson *et al.*, 1974).

It also seems likely that MEC in the dermatoses examined in this study are a manifestation of benign dyskeratosis, since nuclear atypia could not be found in the MEC. More precise understanding of the mechanism of formation of MEC in non-neoplastic dermatoses must be elucidated by further studies.

REFERENCES

- LEVER, W.F. & SCHAUMBURG-LEVER, G. (1975) Bowen's disease. In: *Histopathology of the skin*, 5th edn, p. 473. J.B. Lippincott Company, Philadelphia.
- OFUJI, S. & HORIO, T. (1970) Epidermal multinucleated giant cell in parapsoriasis guttata and eczematous dermatitis. *Acta dermato-venereologica*, **50**, 252.
- OLSON, R.L., NORDQUIST, R.E. & EVERETT, M.A. (1969) Dyskeratosis in Bowen's disease. *British Journal of Dermatology*, **81**, 676.
- OLSON, R.L., GAYLOR, J. & EVERETT, M.A. (1974) Ultraviolet-induced individual cell keratinization. *Journal of Cutaneous Pathology*, **1**, 120.
- OMAR, A., WIESMANN, U.N. & KREBS, A. (1977) Induction of multinucleate cells by 8-MOP and UV treatment *in vitro* and *in vivo*. *Dermatologica*, **155**, 65.
- PATZELT, U. (1926) Zum Bau der menschlichen Epidermis. *Zeitschrift für mikroskopisch-anatomische Forschung*, **5**, 371.
- PINKUS, H. (1951) Examination of the epidermis by the strip method of removing horny layers. I. Observations on thickness of the horny layer, and on mitotic activity after stripping. *Journal of Investigative Dermatology*, **16**, 383.
- SEIJI, M. & MIZUNO, F. (1969) Electron microscopic study of Bowen's disease. *Archives of Dermatology*, **99**, 3.
- SHAHRAD, P. & MARKS, R. (1977) The wages of warmth: changes in erythema ab igne. *British Journal of Dermatology*, **97**, 179.

An immunofluorescence study of pityriasis lichenoides

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SUMMARY

In a study of 27 patients with pityriasis lichenoides IgM and C3 have been observed on direct immunofluorescence of fresh lesions. Other immunoglobulins and complement components were not observed. Immunofluorescence was seen in 31 (72%) of 43 fresh lesions. It occurred in the walls of superficial dermal vessels and along the dermal-epidermal junction. This pattern of immunofluorescence appears to be characteristic of the disease. Uninvolved skin showed the immunofluorescence less frequently and old scaly lesions none. The concept that pityriasis lichenoides is an immune complex disorder is discussed.

Pityriasis lichenoides (PL) is a disorder of obscure aetiology. It is widely accepted to be a vasculitis (Rook *et al.*, 1972), but enzyme histochemical and autoradiographic studies suggest that there is minimal damage to small blood vessel endothelium (Black & Marks, 1972). These authors detected no direct immunofluorescence (IF) in six papules from patients with histologically proven PL. However, IgM, C3 and C1q have been detected by IF in dermal vessel walls of lesions in two patients with acute PL (Hayashi, 1977).

We have reported IF and serological findings in 16 patients which imply that PL is an immune complex (IC) disease (Clayton *et al.*, 1977). The present paper extends our earlier IF observations.

PATIENTS AND METHODS

All 27 patients studied satisfied the criteria for a diagnosis of PL as established by Marks *et al.* (1972). Two patients had the acute form of the disease with blisters. Fresh, papular, non-scaly, purpuric lesions, normal uninvolved skin within an area where lesions were forming and old scaly lesions were examined.

Four millimetre punch biopsies were treated with fluorescein labelled sheep antihuman IgG, M and A and rabbit antihuman C3 as previously described (Clayton *et al.*, 1977). In addition sections were examined for IgD, Clq, C4, properdin and transferrin with optimally diluted goat antihuman

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IgD (Nordic Diagnostics), goat antihuman Clq, C₄, properdin and transferrin (Flow Labs.) and fluorescein labelled rabbit antigoat IgG (Miles Labs.) using an indirect IF technique.

The sections were examined for direct IF in a Leitz Dialux microscope with Ploem incident illumination. The filter system comprised: exciter 2 × KP 490, dichroic mirror TK 510, suppression K515 and edge filter GG 475.

RESULTS

IgM and C₃ immunofluorescence was seen in 31 (72%) of the 43 fresh purpuric lesion biopsies. No IgG, IgA, IgD, Clq, C₄, properdin or transferrin was seen. The IgM and C₃ was in the walls of superficial dermal vessels and along the dermal-epidermal junction (D-EJ) in the dermis. They were not always detected together, nor were they always present both in the vessels and along the D-EJ. The complete picture of IgM and C₃ in the vessels and along the D-EJ was seen in 5 biopsies.

Vessel IF was seen in 25 biopsies (58%)—IgM was detected as commonly as C₃; IgM and C₃ occurred together in 11 biopsies. The fluorescence was in papillary and superficial dermal vessel walls, and granular in appearance often involving only one or two vessels.

D-EJ IF was seen in 21 biopsies (49%)—IgM was detected less frequently than C₃, IgM and C₃ occurred together in 9 biopsies. The fluorescence was seen in the dermis and was granular. On occasion, only single fluorescent granules were distributed intermittently along the D-EJ.

IF, including vessel IF, was seen in all 4 biopsies from the two patients with acute PL. IgM and C₃ were detected in 3 (25%) of the 12 uninvolved skin biopsies. The quality and distribution of IF was similar to that found in the fresh lesions. The 3 old scaly lesions showed no IF.

DISCUSSION

IgM and C₃ have been detected by direct IF both in superficial dermal vessel walls and along the D-EJ in the great majority of biopsies of fresh purpuric lesions of PL. This pattern of IF appears to be characteristic of the disease. Identical findings in acute and chronic PL support the observation that these are morphological variants of the same disease (Marks *et al.*, 1972).

Immunoglobulins and complement components have been demonstrated in dermal vessel walls in a variety of forms of cutaneous vasculitis and they have been taken as evidence of circulating IC disease. Studies of immunoreactant and cell kinetics in serial skin biopsies after histamine injection in patients with leukocytoclastic vasculitis support the contention that deposition of ICs within vessel walls is responsible for the vasculitis (Gower *et al.*, 1977).

Vessel dilatation, said to occur in PL (Black & Marks, 1972), may result in leakage of plasma components and in such circumstances presence of immunoglobulin and complement may have no immunological significance. But we detected no transferrin IF suggesting that this does not account for the observed IF in PL. Leukocytoclasia is not usually seen in PL and blood vessel damage is minimal (Black & Marks, 1972). But we have detected circulating ICs in patients with active PL when IgM and C₃ is detected in the dermal vessel walls of fresh lesions (Clayton *et al.*, 1977). The presence or absence of these ICs correlates with disease activity. An electronmicroscopic study of PL has shown angiitic changes in the periendothelium of small dermal vessels (Pelfini *et al.*, 1976). This would suggest that PL is a manifestation of IC disease and the characteristic mononuclear infiltrate is part of a spectrum of cellular response to deposition of complexes in dermal vessels. This cellular response may depend on circulating complement levels (Soter *et al.*, 1976). Normal blood complement levels as seen in PL (Mowbray & Clayton, 1977) are associated with a predominantly lymphocytic infiltrate. In experimental IC reactions it has been shown that immunoglobulin and

complement disappear 18–48 h after lesion induction (Cream *et al.*, 1971). In our study no IF was seen in biopsies of old scaly lesions. But we experienced technical difficulties in cutting these biopsies and the poor quality of the sections made them difficult to interpret due to intense background IF.

Deposits of immunoglobulin and complement along the D-EJ have been observed in a number of skin disorders, notably lupus erythematosus and pemphigoid. In PL the IF is neither as bright nor extensive as in these conditions but is clearly in the dermis. The finding of the same immunoglobulin and complement component in this area as in the vessel walls suggests that complexes pass through the vessels into the surrounding dermis where they accumulate below the basement membrane.

Hayashi (1977) did not detect a circulating antibody to dermal blood vessel components in his patients. This, together with our finding of IF in some normal uninvolved areas of skin, supports our contention that the ICs play a primary role in PLC and their deposition in vessel walls occurs prior to infiltration of inflammatory cells and production of the characteristic papule. There may be an optimal quantity of deposited IC necessary to produce a lesion.

REFERENCES

- BLACK, M.M. & MARKS, R. (1972) The inflammatory reaction in pityriasis lichenoides. *British Journal of Dermatology*, **87**, 533.
- CLAYTON, R.J., HAFFENDEN, G., DU VIVIER, A., BURTON, J. & MOWBRAY, J. (1977) Pityriasis lichenoides—an immune complex disease. *British Journal of Dermatology*, **97**, 629.
- CLAYTON, R.J. & MOWBRAY, J. (1977) Unpublished.
- CREAM, J.J., BYCESON, A.D.M. & RYDER, G. (1971) Disappearance of immunoglobulin and complement from the arthus reaction and its relevance to studies of vasculitis in man. *British Journal of Dermatology*, **84**, 106.
- HAYASHI, T. (1977) Pityriasis lichenoides varioliformis acuta; immunopathologic study. *Journal of Dermatology*, **69**, 477.
- MARKS, R., BLACK, M.M. & WILSON JONES, E. (1972) Pityriasis lichenoides: a reappraisal. *British Journal of Dermatology*, **86**, 215.
- PELFINI, C., CALLIGARO, A. & SERRI, F. (1976) Parapsoriasi Guttata, Una Angiopatia, Rilievi Ultrastrutturali. *Giornale Italiano di Dermatologia e Minerva Dermatologica.*, **111**, 132.
- ROOK, A., WILKINSON, D.S. & EBLING, F.J.G. (1972) *Textbook of Dermatology*, p. 604. Blackwell Scientific Publications Oxford.
- SOTER, N.A., MIHM, M.C., JR, GIGLI, I., DVORJAK, H.F. & AUSTEN, K.F. (1976) Two distinct cellular patterns in cutaneous necrotizing angiitis. *Journal of Investigative Dermatology*, **66**, 344.

Complement and immunoglobulin deposits in the skin of patients with atopic dermatitis

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SUMMARY

The immunofluorescent patterns of uninvolved and involved skin biopsies from eight patients with atopic dermatitis were studied, using direct immunofluorescence techniques to identify deposits of the immunoglobulins G, A and M as well as the complement factors C1q, C3, C4, C5, factor B and properdin. Immunoglobulin deposits (mainly IgG) were found in five patients, complement deposits in three patients in the basement membrane zone. In three patients the immunofluorescence was positive for C3, in two patients for C1q, C4 and C5. Regarding the factors of the alternative pathway of the complement system, two patients showed deposits of properdin, one of factor B.

The changes were not confined to the eczematous lesions, but were found in uninvolved skin too. The most prominent changes were observed in patients with severe disease.

Immunological mechanisms have long been invoked to explain the pathogenesis of atopic dermatitis (Wüthrich *et al.*, 1972; Braun-Falco & Burg, 1974; Hanifin & Lobitz, 1977). The aetiology of this common skin disease, however, remains still largely obscure. Among the abnormalities of the immune system observed in patients with atopic dermatitis are increased serum IgE levels (Berg & Johansson, 1969; Stone *et al.*, 1976) and evidence for decreased cellular immunity (Lobitz *et al.*, 1972; McGeady & Buckley, 1975; Rachelefsky *et al.*, 1976).

Relatively little is known about the role of complement and vasoactive mediators in this dermatological condition. We recently reported results of a preliminary study, in which we found abnormal levels of complement and histamine in the plasma of patients with atopic dermatitis (Ring *et al.*, 1978).

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The presence of immunoglobulin and complement-bearing lymphocytes in atopic eczema has been reported by Cormane *et al.* (1974) and recently by Hodgkinson *et al.* (1977). The latter group found a high percentage of positive immunofluorescence with anti-C₃ among these patients; however, positive readings were observed in controls as well.

The aim of this study was to look for deposits of immunoglobulins and of complement components, both from the classical and the alternative pathway, in the eczematous lesions and in the uninvolved skin of patients suffering from atopic dermatitis.

PATIENTS AND METHODS

Five male and three female patients between 2 and 45 years of age suffering from atopic dermatitis were investigated. Informed consent was obtained either from the patients or from their parents prior to examination and skin biopsy. The eczema was diagnosed as atopic dermatitis when four of the following six criteria were positive: onset of the eczema in childhood; positive history of allergies; positive family history of allergies; high serum IgE; typical localization (extensor surfaces in young children, predilection toward flexural surfaces later on); no evidence of contact dermatitis.

Seven healthy volunteers with no personal or family history of eczema, asthma, hay fever or any other dermatological illness served as controls.

Clinical Evaluation. The severity of the disease was evaluated according to a scoring system developed by Clendenning *et al.* (1973) which rates from 0 to 4 each of the following symptoms: Erythema, oedema, vesiculation, crusting, excoriation, scaling, lichenification, pigmentation, itching and loss of sleep. In this scoring system an infinitely severe skin inflammation would score a 'dermatitis index' of 40 points. Also evaluated was the extent of the eczema, estimated as the percentage of the body surface involved.

Serology. Heparinized blood was taken for immunochemical analysis. The serum levels of IgG, IgM, IgA and C₃ were assayed by the radial immunodiffusion technique of Mancini *et al.* (1965). Serum IgE was measured by using the paper-radio-immunosorbent-test (PRIST) (Pharmacia Diagnostics, Uppsala, Sweden).

Skin samples. Three mm punch biopsies were taken from involved and uninvolved skin sites, mounted in OCT medium (Miles Laboratories, Naperville, Illinois), snapfrozen and stored at -70°C until analyzed.

Immunofluorescence technique. For the immunofluorescence studies the direct technique was used. Four micron sections were incubated with fluorescein isothiocyanate (FITC)-labelled antisera. Washing was done in phosphate buffered saline (pH 7.4) (PBS). The slides were read in an Ortholux II fluorescence microscope. All sections were assessed 'blind'; each deposit pattern was graded as negative, 1+, 2+ or 3+.

Preparation of antisera. FITC-labelled anti-human IgG, anti-human IgA and anti-human IgM were purchased from Meloy Laboratories (Springfield, Virginia). FITC-antisera against the complement components C_{1q}, C₄, C₃, C₅, factor B and properdin were prepared in our laboratory using monospecific antisera to the single proteins and a previously described conjugation technique (Wilson *et al.*, 1976; Arroyave & Tan, 1976).

RESULTS

Clinical data. The most important clinical characteristics of our patients are summarized in Table 1. All patients had a positive personal history of allergy, four a positive family history. At the time of the investigation only one patient (No. 4) was receiving systemic steroid medication in the form of 20 mg prednisolone/day. The dermatitis indices ranged from 8 to 20 points, the percentage of body surface involved from 5 to 50%.

Immunoglobulin deposits. Skin samples from five of the eight patients showed deposits of immunoglobulins at the dermal-epidermal junction. Most deposits were IgG (see Table 2). One patient showed IgM (No. 3) and another patient IgA and IgM (No. 7).

None of the controls showed immunoglobulin deposits in the direct immunofluorescence. Two patients showed immunoglobulin deposits only in the involved skin (Nos. 3 and 4), whereas in three other patients immunoglobulin deposits were found in uninvolved skin as well (Nos. 5, 7 and 8).

Complement deposits. Complement deposits were found in three of the eight patients (Table 3), generally in the basement membrane zone. All three patients showed deposits of complement factor

TABLE 1. Clinical data of patients investigated

Patient no.	Sex	Age	Steroid Treatment (week before exam)	Allergy History	
				Personal	Family
1	M	28	Topical	Wool, asthma	—
2	M	2	—	Milk	—
3	F	7	Topical	Dogs, hay fever	+
4	M	45	20 mg Prednisolone/day	Pollen, asthma	—
5	F	15	—	Cats	+
6	M	6	Topical	Wool, asthma	+
7	F	33	Topical	Wool, pollen, drugs	+
8	M	15	—	Pollen, asthma	—

TABLE 2. Immunoglobulin deposits in the skin of atopic dermatitis patients*

Patient No.	Uninvolved skin			Involved skin		
	IgG	IgM	IgA	IgG	IgM	IgA
1	—	—	—	—	—	—
2	—	—	—	—	—	—
3	—	—	—	—	+	—
4	—	—	—	+	—	—
5	+	—	—	++	—	—
6	—	—	—	—	—	—
7	+	±	+	+	—	—
8	+	—	—	+	—	—

* + = positive; — = negative.

TABLE 3. Complement deposits in the skin of atopic dermatitis patients*

Patient no.	Uninvolved skin						Involved skin					
	C1q	C3	C4	C5	Factor B	Properdin	C1q	C3	C4	C5	Factor B	Properdin
1	—	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—
5	+	+	+	+	—	—	+	+	+	+	—	+
6	—	—	—	—	—	—	—	—	—	—	—	—
7	+	+	+	+	+	+	—	—	+	—	+	+
8	—	+	—	—	—	—	—	+	—	—	—	—

* += positive; — = negative.

C3 in either involved or uninvolved skin, two patients showed deposits of factors C1q, C4 and C5 as well. The latter two also showed deposits of alternative pathway products of the complement system. Properdin was found in patients Nos. 5 and 7 (Fig. 1), and properdin and factor B in patient No. 7 in involved as well as in uninvolved skin.

Serology. Plasma levels of IgG, IgM and IgA were within normal limits in all patients except patient no. 4, in whom IgG and IgM were slightly elevated. All patients had elevated serum IgE concentrations. In four patients plasma C3 levels were slightly decreased.

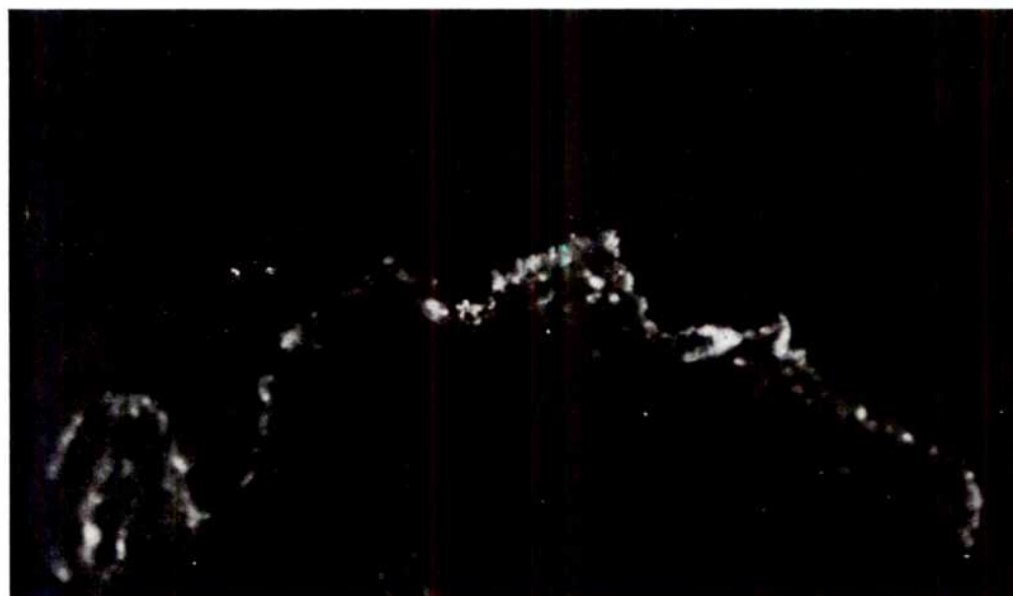


FIGURE 1. Immunofluorescence of antiserum to properdin noted at the dermal-epidermal junction (patient No. 5, original magnification $\times 300$).

TABLE 4. Correlation between immunofluorescence findings and clinical severity of atopic dermatitis

	Dermatitis index (points in severity scale)	Body surface involved %
Patients with negative immunofluorescence (n=3)	10, 8, 8	5, 10, 5
Patients with positive immunofluorescence for immunoglobulins (n=5)	10, 14, 10, 14, 22	20, 25, 5, 40, 50
Patients with positive immunofluorescence for complement components (n=3)	10, 14, 22	5, 40, 50

Correlation of immunofluorescence patterns and clinical severity: An attempt to correlate the presence of abnormal immunofluorescence findings with clinical severity of the disease is shown in Table 4. The five patients with positive immunofluorescence findings generally showed a higher severity score when compared to the three patients with negative staining.

DISCUSSION

In this study we found deposits of immunoglobulin and complement factors in the skin of several patients suffering from atopic dermatitis.

Immunoglobulin and complement C3 deposits have been described in a variety of dermatoses such as systemic lupus erythematosus (SLE) (Beutner *et al.*, 1970; Tan, 1976; Arroyave *et al.*, 1976), psoriasis (Braun-Falco *et al.*, 1977), dermatitis herpetiformis, pemphigus, bullous pemphigoid and others (Provost & Tomasi, 1973; Seah *et al.*, 1973). In SLE, IgG deposits are most commonly found (Tan, 1976), generally in a 'lumpy' distribution. IgA is found in almost 100% of the cases of dermatitis herpetiformis (Seah *et al.*, 1973). Hodgkinson *et al.* (1977) found IgG deposits in 7 out of 14, IgM in 9 out of 14 and IgA in 4 out of 14 eczematous lesions of atopic dermatitis.

Generally, the pattern of immunofluorescence in our patients was found to be in the dermal-epidermal junction in a patchy linear, sometimes lumpy distribution, reminiscent in some cases of the pattern observed in SLE (Tan, 1976). This would suggest a possible presence of immune complexes in this area.

We found C3 deposits in only 3 of the 8 patients, whereas Hodgkinson *et al.* (1977) described them in 12 out of 14 biopsies of eczematous lesions and in 6 out of 7 uninvolved skin areas. This might be explained by the antisera used. Hodgkinson *et al.* (1977) found C3 deposits in a high number of normal volunteers, too. In our control population we never were able to demonstrate C3 deposits in skin biopsies.

Interestingly, the C3 deposits were accompanied by deposition of elements of both the classical (C1q, C4) and the alternative complement pathway (factor B, properdin), in two of our patients in eczematous as well as uninvolved skin.

The question regarding the origin of the immunoglobulin and complement deposits observed

cannot be answered by this study. It may be speculated, however, that they are formed locally from immunoglobulin and complement produced in the skin, as Lai A Fat (1974) found in studying the skin of five patients with atopic dermatitis. This hypothesis is further supported by the observation of Hodgkinson *et al.* (1977) who found that the amount of immunoglobulin and complement C₃ deposits in the skin correlated with the number of immunoglobulin and C₃-positive lymphocytes in the dermal papillae.

On the other hand, deposition of immunoglobulin and complement may simply reflect circulating plasma concentrations of the various proteins diffusing passively into the extravascular compartment and binding to receptors present in the skin, as happens for example, when massive doses of allo-geneic IgG are injected intravenously in dogs (Ring *et al.*, 1978). However, the absence of gross abnormalities in serum levels of IgG, IgM and IgA among our patients, even those with Ig deposits, leads us to suspect that such mechanisms do not primarily produce the changes observed.

Since immunoglobulins and complement were deposited with almost equal frequency in clinically involved as well as uninvolved skin, these deposits probably do not represent 'unspecific' inflammatory changes resulting from increased capillary permeability.

Taken together, the results of this preliminary study suggest that immune processes involving both classical and alternative pathway complement components might play a role in the pathophysiology of atopic dermatitis.

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REFERENCES

- ARROYAVE, C.M. & TAN, E.M. (1976) Detection of complement activation by counter immunoelectrophoresis (CIE). *Journal of Immunological Methods*, **13**, 101.
- ARROYAVE, C.M., WILSON, M.R. & TAN, E.M. (1976) Serum factors activating the alternative complement pathway in autoimmune disease: Description of two different factors from patients with systemic lupus erythematosus. *Journal of Immunology*, **116**, 821.
- BERG, T. & JOHANSSON, S.G.O. (1969) IgE concentration in children with atopic diseases. *International Archives of Allergy and Applied Immunology*, **36**, 219.
- BEUTNER, E.H., CHORZELSKI, T.P. & JORDON, R.E. (1970) *Autosensitization in Pemphigus and Bullous Pemphigoid*. Charles C. Thomas, Springfield, Illinois.
- BRAUN-FALCO, O. & BURG, G. (1974) Zelluläres Infiltrat und Kapillaren bei Neurodermitis diffusa. Quantitativ enzymzytochemische und histochemische Untersuchungen. *Archives of Dermatological Research*, **249**, 113.
- BRAUN-FALCO, O., MACIEJEWSKI, W., SCHMOECKEL, C. & SCHERER, R. (1977) Immunoelectronmicroscopical demonstration of *in vivo* bound complement C₃ in perioritic lesions. *Archives of Dermatological Research*, **260**, 57.
- CLENDENNING, W.E., CLACK, W.E., OGAWA, M. & ISHIZAKA, K. (1973) Serum IgE studies in atopic dermatitis. *Journal of Investigative Dermatology*, **61**, 233.
- CORMANE, R.H., HUSZ, S. & HAMERLINCK, F. (1974) Immunoglobulin and complement-bearing lymphocytes in allergic contact dermatitis and atopic dermatitis (eczema). *British Journal of Dermatology*, **90**, 597.
- HANIFIN, J.M. & LOBITZ, W.C. (1977) Newer concepts of atopic dermatitis. *Archives of Dermatology*, **113**, 663.
- HODGKINSON, G.I., EVERALL, J.D. & SMITH, H.V. (1977) Immunofluorescent patterns in the skin in Besnier's prurigo. *British Journal of Dermatology*, **96**, 357.
- LAI A FAT, R.F.M., CORMANE, R.H. & VAN FURTH, R. (1974) An immunohistopathological study on the synthesis of immunoglobulins and complement in normal and pathological skin and the adjacent mucous membrane. *British Journal of Dermatology*, **90**, 123.
- LOBITZ, W.E., HONEYMAN, J.F. & WINKLER, N.W. (1972) Suppressed cell-mediated immunity in two adults with atopic dermatitis. *British Journal of Dermatology*, **86**, 317.

- MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.
- MCGEADY, S.J. & BUCKLEY, R.H. (1975) Depression of cell-mediated immunity in atopic eczema. *Journal of Allergy and Clinical Immunology*, **56**, 393.
- PROVOST, T.T. & TOMASI, T.B., JR (1973) Evidence for complement activation via the alternate pathway in skin diseases. I. Herpes gestationis, systemic lupus erythematosus and bullous pemphigoid. *Journal of Clinical Investigation*, **52**, 1779.
- RACHELEFSKY, G.S., OPELZ, G., MICKEY, M.R., KIUCHI, M., TERASAKI, P.I., SIEGEL, S.C. & STIEHM, E.R. (1976) Defective T cell function in atopic dermatitis. *Journal of Allergy and Clinical Immunology*, **57**, 569.
- RING, J., DUSWALD, K.H., BACHMANN, T., STEPHAN, W. & BRENDDEL, W. (1977) Konzentration intravenös applizierter Gammaglobulin-Präparate in der Hundehaut. *Archives of Dermatological Research*, **260**, 201.
- RING, J., SENTER, T., ARROYAVE, C.M., CORNELL, R. & TAN, E.M. (1978) Role of complement and histamine in atopic dermatitis. *Clinical Research*, **26**, 210.
- SEAH, P.P., FRY, L., MAZAHARI, M.R., MOWBRAY, J.F., HOFFBRAND, A.V. & HOLBORROW, E.J. (1973) Alternate-pathway complement fixation by IgA in the skin in dermatitis herpetiformis. *Lancet*, **i**, 175.
- STONE, S.P., GLEICH, G.J. & MULLER, S.A. (1976) Atopic dermatitis and IgE. Relationship between changes in IgE levels and severity of disease. *Archives of Dermatology*, **112**, 1254.
- TAN, E.M. (1976) Immunopathology and pathogenesis of cutaneous involvement in systemic lupus erythematosus. *Journal of Investigative Dermatology*, **67**, 360.
- WILSON, M.R., ARROYAVE, C.M., NAKAMURA, R., VAUGHAN, J. & TAN, E.M. (1976) Activation of the alternative complement pathway in systemic lupus erythematosus. *Clinical and Experimental Immunology*, **26**, 11.
- WÜTHRICH, B., STORCK, H., GROB, P. & SCHWARZ-SPECK, M. (1972) Zur Immunpathologie der Neurodermitis. *Archives of Dermatological Research*, **244**, 327.

Dermo-epidermal relationships in pyogenic granulomata

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SUMMARY

The epidermis of pyogenic granulomata is presented with an unusual problem of distorted dermo-epidermal relationships. In this study we have attempted to delineate the problem and have studied the way in which the epidermis copes with its difficulties.

Sixteen pyogenic granulomata with (where possible) paralesional skin (and for comparison ten Campbell de Morgan spots) were studied by cell kinetic techniques. High labelling indices were found in the epidermis and in the endothelium in the pyogenic granulomata (contrasting strikingly with the findings in the Campbell de Morgan spots). A variable morphological response was found ranging from hyperplasia with parakeratosis to epidermal thinning and atrophy. Possible mechanisms for the epidermal changes are discussed and it is suggested that the data produced from this and similar studies may aid the understanding of dermo-epidermal interaction in a variety of common skin disorders including psoriasis.

The dramatically rapid expansion of pyogenic granulomata (PG) is a striking clinical phenomenon. Paradoxically their growth rate often exceeds that seen in cutaneous malignancies. However, they rarely reach more than 2 cm in diameter and do not become truly malignant, although the development of satellite lesions is well documented (Evans, 1954; Frain-Bell, 1958; Warner & Wilson Jones, 1968). Such a rapid expansion in the dermal endothelial mass poses particular problems for the overlying epidermis and in this study we have attempted to relate epidermal growth with that of the underlying endothelial mass. In order to determine the role of tissue expansion on the observed epidermal changes, more slowly growing benign vascular lesions—Campbell de Morgan spots (CMS)—were also studied.

METHODS

Sixteen patients with PG were studied. The patients' age and sex, the anatomical site and duration of the lesions as well as the presence or absence of erosion are noted in Table 1. All lesions were completely excised including wherever possible a surrounding zone of 'normal' perilesional skin. Thin

TABLE 1. Clinical details

Patient			P.G.		
Patient no.	Age	Sex	Anatomical site	Duration (weeks)	Erosion
1	17	M	Chest	4	Absent
2	40	M	Left ring finger	4	Present
3	15	F	Right shoulder	8	Absent
4	27	F	Right ring finger	4	Present
5	52	M	Left palm	16	Present
6	46	M	Scalp	12	Absent
7	29	F	Back of neck	8	Present
8	29	F	Right index finger	6	Present
9	23	F	Left upper eyelid	3	Absent
10	11	M	Left scapular area	3	Present
11	15	M	Back	3	Present
12	30	F	Right index finger	8	Present
13	58	F	Occiput	10	Absent
14	23	F	Left shoulder	16	Absent
15	32	M	Back	4	Present
16	76	M	Chin	24	Absent

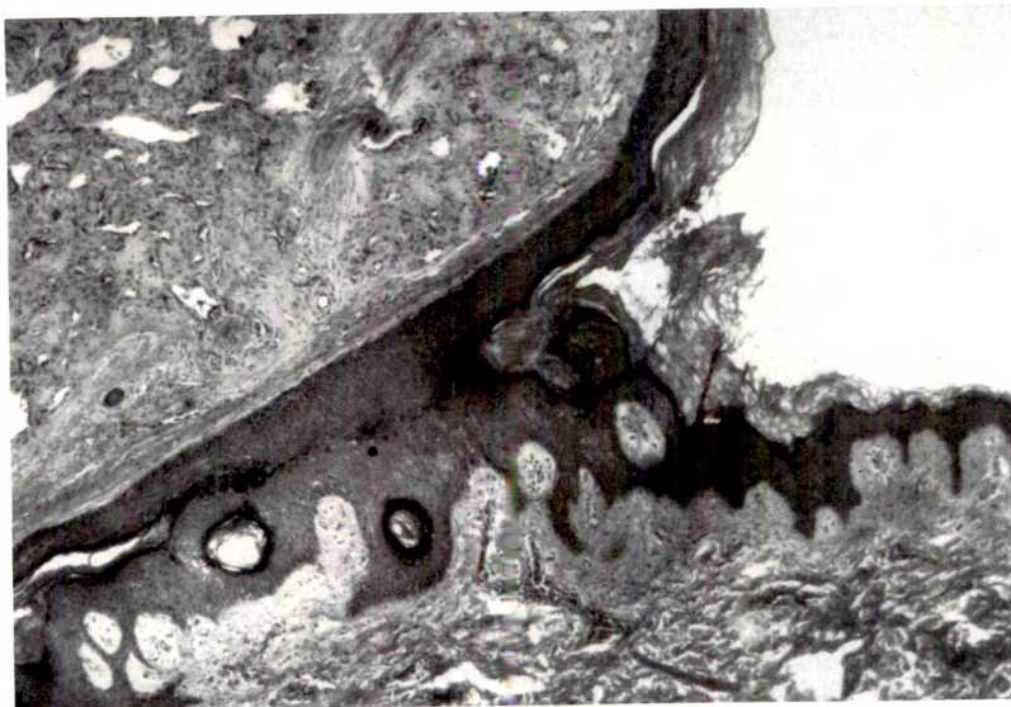


FIGURE 1. Edge of a PG showing differences in rete pattern between lesional and paralesional epidermis and the 'collar' effect (H&E, $\times 120$).

(1 mm) slices of the lesions were incubated in Eagle's minimal essential medium in disposable Petri dishes at 37°C in an atmosphere of 5% CO₂ and 95% air for 2.5 h using a shaking incubator. Tritiated thymidine (specific activity, 18 Ci/mmol) was added to the medium at a concentration of 1 µCi/ml.

After incubation the tissue slices were fixed in 10% formalin and paraffin sections were prepared histologically according to routine methods. Dewaxed paraffin sections were dipped in nuclear emulsion (Ilford K2), exposed in dark boxes at 4°C for 2 week periods and developed in Kodak D19 developer. The autoradiographs so obtained were stained with haematoxylin and eosin and examined microscopically. The thickness of the epidermis of the lesional and paralesional skin excluding the 'collar' (Figs 1 and 5) was measured by counting the cells in the viable epidermis in a plane perpendicular to the surface at 20 randomly chosen points (mean cell number, MCN). The degree of rete hypertrophy was assessed by measuring the ratio of the length of the basal cell layer to the length of the granular cell layer—the B/G ratio. The presence and extent of parakeratosis and/or erosion were recorded.

The numbers of epidermal cells in the premitotic DNA synthesis phase were determined by counting the numbers of labelled basal and suprabasal cells in those areas that were uniformly labelled per 400–800 total basal epidermal cells in both lesions and paralesional epidermis and expressing the result as a percentage (Labelling Index-LI). Where possible data were obtained for several areas of the epidermis in the lesion. Endothelial LI's were determined by counting the numbers of labelled endothelial cells and the total number of endothelial cells in a minimum of 5 high power fields and expressing the result as a percentage.

In addition three PG's were 'double labelled' using the method of Heenen & Galand (1971) in order to determine the length of the DNA synthesis (S) phase for the involved epidermis. The tissue was exposed during incubation to two pulses of tritiated thymidine (specific activities 2 Ci/mmol followed by 18 Ci/mmol) each of 15 min and separated by a period of 60 min when the tissue was incubated in unlabelled Eagle's medium. From the resultant autoradiographs histograms were constructed of the numbers of labelled epidermal cells with differing grain counts. Labelled cells were largely of two populations, weakly labelled and heavily labelled, the dividing line occurring at 30–40 grains per nucleus.

Using the relationship:

$$\frac{N_h}{N_w} = \frac{S}{t} \text{ (Wegener, Hollweg & Maurer, 1964)}$$

where N_h and N_w are the numbers of heavily and weakly labelled cells respectively, t the time in hours between the pulses of tritiated thymidine, the length of the DNA synthetic (S) phase in hours could be calculated.

Ten longstanding CMS lesions from hospital inpatients being treated for a variety of skin disorders were excised with a rim of perilesional skin and slices were incubated in Eagle's medium containing tritiated thymidine (specific activity 18 Ci/mmol) as described above. Autoradiographs were prepared and studied in an identical manner.

RESULTS

The histometric data for the epidermis of the lesions and paralesional skin are shown in Table 2. In four lesions the epidermis was completely eroded. A further four PG's showed partial erosion histologically. A variable degree of parakeratosis was seen in 7 PG's. Neither erosion nor parakeratosis was seen in the paralesional epidermis. The effacement of the rete pattern in the lesional epidermis (B/G

TABLE 2. Epidermal histometric data (no. of + 's represents on an arbitrary scale the extent of parakeratosis and erosion)

Pt. no.	Lesional				Paralesional			
	MCN	B/G ratio	Extent of parakeratosis	Extent of erosion	MCN	B/G ratio	Extent of parakeratosis	Extent of erosion
1	5.0	1.00	++	—	4.9	1.28	—	—
2	4.9	1.00	+++	—			—	—
3	4.6	1.00	++	—	4.7	1.60	—	—
4	5.4	1.00	++	+			—	—
5		Completely eroded		+++			—	—
6	5.1	1.00	—	—			—	—
7		Completely eroded		+++	6.7	2.45	—	—
8		Completely eroded		+++			—	—
9	5.4	1.00	—	—	6.1	1.35	—	—
10	4.8	1.00	++	+	4.7	1.25	—	—
11	3.3	1.00	+++	++	5.8	1.45	—	—
12		Completely eroded		+++			—	—
13	6.1	1.00	—	—			—	—
14	5.5	1.01	—	—	4.9	1.32	—	—
15	4.0	1.00	+++	+	7.8	1.62	—	—
16	5.7	1.01	—	—	5.4	1.01	—	—
Mean	5.0	1.00			5.7	1.48		
s.d.	± 0.76	± 0.00			± 1.06	± 0.41		

ratio 1.00) is in marked contrast to its accentuation in the acanthotic paralesional areas (B/G ratio 1.48 ± 0.41) (Figs 1 and 5).

The autoradiographic findings were striking, both epidermal and endothelial compartments showing considerable numbers of labelled cells (Figs 2 and 3). The great majority of labelled epidermal cells of the lesions were basal whereas in the relatively acanthotic paralesional areas labelled suprabasal cells were more frequent. Although the paralesional epidermis tended to be fairly uniformly labelled, the epidermis in the lesions showed variations in the density of labelled cells. Table 3 gives details of LI, MCN and type of keratinization in the non-eroded lesional epidermis of 12 PG's. It can be seen that parakeratosis was strongly associated with high LI's (32.4 ± 9.0) in contrast to orthokeratosis (17.0 ± 7.7).

Overall there was an inverse relationship between the LI of the epidermis of the lesions and MCN ($r = 0.49$; $0.05 > P > 0.02$). Variations in the density of labelled cells were also seen in the endothelial compartment not only between lesions but also within different parts of the same PG.

Epidermal and endothelial LI's are given in Table 4 and are in marked contrast to the data obtained for the CMS (Table 5). The double labelling technique gave 'S' phase durations of 6.3, 8.9, and 8.3 h for the lesional epidermis of three PG's. There was a positive correlation ($r = 0.705$; $0.02 > P > 0.01$) between the LI's for the endothelial mass and the lesional epidermis (Fig. 4). There was no correlation between the duration of the PG's and their LI's.

Fig. 5 is a diagrammatic representation of a typical PG and portrays several features already described. Where erosion was present the surrounding epidermis almost invariably showed parakeratosis. Furthermore, these parakeratotic areas often tended to be thinner than the adjacent orthokeratotic

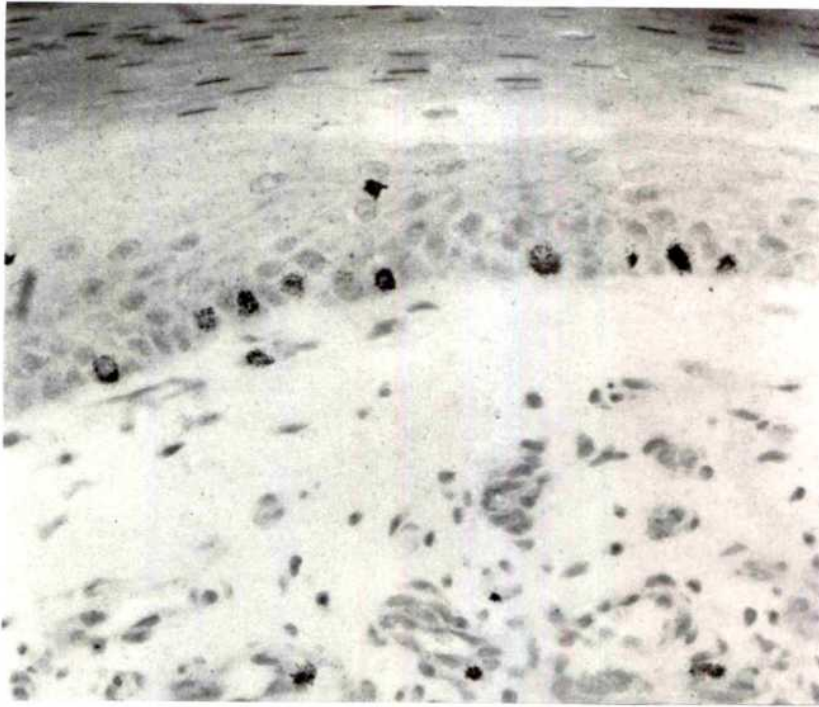


FIGURE 2. Autoradiograph of PG epidermis showing labelled basal cells and overlying parakeratosis (H&E, $\times 400$).

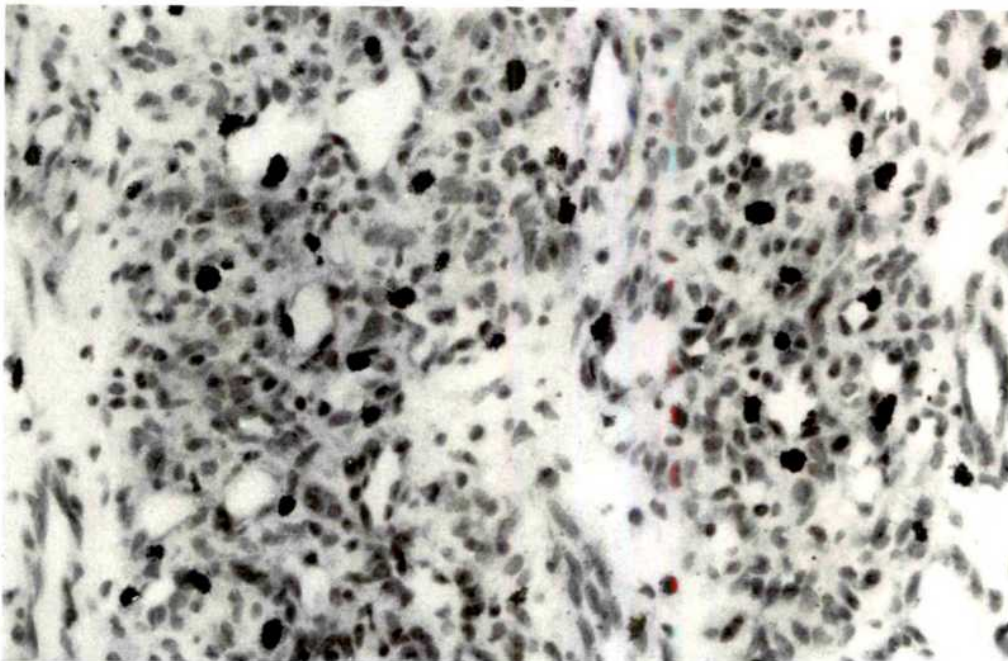


FIGURE 3. Autoradiograph of a PG endothelial mass showing the high frequency of labelled endothelial cells (H&E, $\times 400$).

TABLE 3. Details of LI, MCN and type of keratinization in non-eroded lesional epidermis

PG no.	LI (%)	MCN	Type of keratinization
1	28	4.4	Parakeratosis
	25	4.1	Parakeratosis
	25	4.3	Parakeratosis
	12.5	7.1	Orthokeratosis
2	32	4.9	Parakeratosis
3	35	3.9	Parakeratosis
	35	4.1	Parakeratosis
	18.6	5.9	Orthokeratosis
4	46.8	4.8	Parakeratosis
	28.4	5.9	Orthokeratosis
6	11.3	5.1	Orthokeratosis
9	23.8	5.4	Orthokeratosis
10	12.6	4.2	Orthokeratosis
	33	5.3	Parakeratosis
11	45.8	3.3	Parakeratosis
13	29.7	6.1	Orthokeratosis
14	14.2	5.5	Orthokeratosis
15	18.2	4.0	Parakeratosis
16	9.3	5.2	Orthokeratosis
	9.3	6.1	Orthokeratosis

TABLE 4. Epidermal and endothelial labelling indices

PG no.	Epidermis		Endothelium
	Lesional (%)	Paralesional (%)	Endothelium (%)
1	22.6	5.7	15.8
2	32.0	—	17.8
3	29.5	6.1	12.1
4	37.6	—	12.8
5	Completely eroded	—	17.0
6	11.3	—	8.5
7	Completely eroded	40.9	13.0
8	Completely eroded	—	19.8
9	23.8	15.6	14.0
10	22.8	4.1	9.8
11	45.8	10.6	15.3
12	—	—	12.1
13	29.7	—	9.9
14	14.2	7.9	5.4
15	18.2	15.0	11.5
16	9.4	7.5	4.6
Mean	24.7	13.0	12.5
s.d.	± 10.8	± 12.8	± 4.2

TABLE 5. Epidermal histometric and autoradiographic data for CMS

	Epidermis		
	Lesional	Paralesional	Endothelium
MCN	3.6 ± 0.44	3.6 ± 0.38	—
B/G ratio	1.04 ± 0.03	1.12 ± 0.03	—
LI	6.24 ± 1.35	6.21 ± 1.40	0.97 ± 0.18

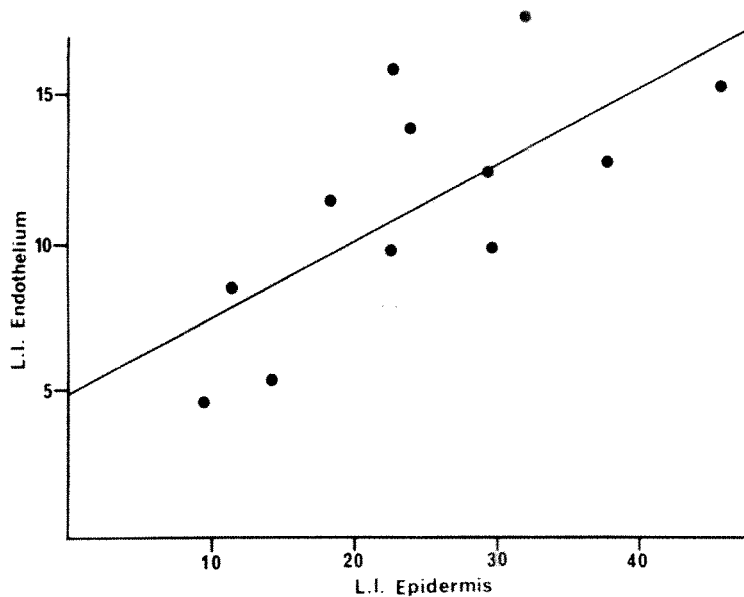


FIGURE 4. Graph showing the relationship between epidermal and endothelial LI's for PG's.
 $y = 0.26x + 4.93$, $r = 0.71$.

zones. Although a progressively thinner margin with parakeratosis was the most usual boundary of an eroded area, some lesions did show areas of epidermis with a variable degree of hyperkeratosis undergoing necrosis and erosion. One other frequently noted feature both clinically and histologically was the presence of a 'constricting' epidermal collar and this appearance together with the complete effacement of the rete pattern in many lesions suggested an apparent origin for the PG from one or two dermal papillae.

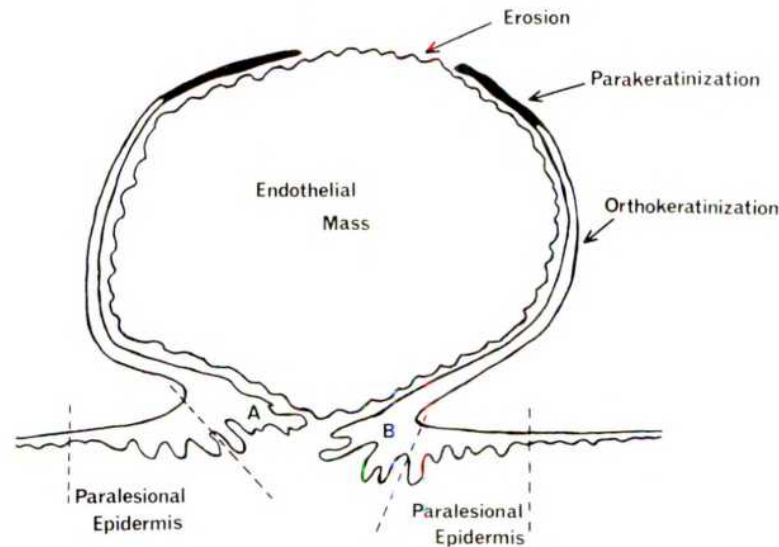


FIGURE 5. Diagrammatic representation of a 'typical' PG. A and B form part of the epidermal 'collar'.

DISCUSSION

This study demonstrates that the rapid growth of PG lesions observed clinically is probably on the basis of cellular growth, although it does not exclude an additional component due to the accumulation of oedema and/or inflammatory cells. Thus, both epidermal and endothelial compartments are marked by high LI's probably indicating an increased rate of cell division. The epidermal 'S' phase lengths for the 3 'double labelled' PG's fell within the range for normal epidermis (5.9 h (Christophers & Schaumloffel, 1967) to 11.5 h (Chopra & Flaxman, 1974)). Although there is inherent in this method a problem sometimes in distinguishing between weakly and heavily labelled cells we feel that our figures for 'S' phase duration are valid. It should be noted that the technique we have employed is by no means ideal for determining 'S' phase lengths. It represents a compromise and has been employed more in order to obtain directional information than to provide precise values. Since, therefore, 'S' phase is not prolonged, the increased LI's are probably a true reflection of increased epidermal mitotic activity. It has been said that as the epidermal mitotic rate increases in man the 'baseline' tends to become more folded due to crowding of the basal layer, i.e. the B/G ratio increases as in psoriasis (Mitrani, 1978). However, there are at least two situations characterized by increased epidermopoiesis, where this does not happen. Firstly, where the basal cell population is depleted by erosion as in lichen planus (Marks, Black & Wilson Jones, 1973) and discoid lupus erythematosus (Davies & Marks, 1977) and secondly in PG's where as this study shows very low B/G ratios are the rule. It should also be noted that a highly folded dermo-epidermal junction may not be characterized by a high rate of cell division, as for example in seborrhoeic warts (Lachapelle, 1969). In PG the low B/G ratio may be the result of rapid underlying dermal expansion tending to 'stretch' the epidermis and consequently preventing basal crowding despite high LI's. However, the CMS epidermis also showed considerable effacement of the rete pattern as compared with the paralesional areas although there was no difference in LI between these two sites. It seems more likely that the loss of rete pattern is due to the origins of both PG and CMS from single dermal papillae.

In contrast to the low B/G ratios in the epidermis of PG's the immediately paralesional epidermis showed moderate acanthosis, increased B/G ratios and often elevated LI's, features of a hyperplastic response.

With regard to the dermis the PG endothelial LI's were strikingly high (12.5 ± 4.2), in marked contrast to that obtained with CMS lesions (0.97 ± 0.18) and in the endothelial cells of rosacea (1.3 ± 0.3) (Marks, 1973). Often, however, the endothelial labelling in individual PG's was not uniform. Although in part this might have been due to uneven penetration of the incubated slices by the tritiated thymidine, we feel a more important explanation is the presence of differing endothelial cell maturation even within the same lesion (Davies, Atai & Marks, 1978).

The lack of correlation between the duration of PG's and the corresponding LI of the epidermis (or endothelium) is in accord with the clinical behaviour of these lesions. Close questioning of the patients often reveals that the growth rate of their PG's is somewhat irregular and certainly not linear.

Given that the endothelial proliferation and expansion is the primary event in PG's, how are the epidermal changes mediated? It is tempting to postulate that some, at least, of the increased lesional epidermal mitotic activity is secondary to the rapid expansion of the endothelial mass. Francis & Marks (1977) have shown that rapid 'non-inflammatory' stretching of the skin increases the epidermal LI but that if no further 'stretch' occurs the situation rapidly stabilizes. An alternative (or additional) explanation might be that the endothelial mass releases a chemical mediator which stimulates epidermopoiesis and we are currently investigating this possibility. Although the parakeratosis observed may be interpreted as a direct consequence of increased rate of epidermal cell division and decreased transit times, the explanation for the epidermal erosion in PG's is more elusive. On theoretical grounds it can be easily calculated that the epidermis is more than capable of 'keeping up' with any rapidly expanding dermal mass. For example if one considers a very rapidly growing spherical PG reaching a diameter of 2 cm in 4 weeks and the average keratinocyte having surface area of $100 \mu\text{m}^2$ it can be easily calculated that, assuming an epidermal MCN of 5 cells, the PG will have approximately 65×10^6 epidermal cells. If one assumes a cell cycle time of 48 h it can be shown that this cell population could in theory be derived from approximately 4000 germinative cells (i.e. 0.006% of the eventual number of epidermal cells). Since the percentage of original germinative cells is almost certainly many times larger than this it can be seen that erosion on the basis of insufficient keratinocytes is extremely improbable. Ischaemic necrosis of the lesional epidermis due to thrombosis of areas of the endothelial mass is a probable cause of erosion in some lesions and in this context Ryan (1978) has failed to detect any fibrinolytic activity in PG endothelium. A further important factor is the very high metabolic demands of a rapidly dividing epidermis and this in conjunction with a tendency to endothelial thrombosis could lead to a point beyond which epidermal infarction and erosion might follow. Additional factors causing erosion in PG's would be the 'proud' nature of the lesions together with the friability of the immature epidermis.

So far the discussion has been concerned only with the mitotic activity as the means by which the endothelial mass is invested in epidermis. Active epidermal cell movement and migration are probably also of importance and it is of interest to note that labelled suprabasal cells were common in the paralesional areas but not in the lesional epidermis. This is probably a reflection of the plane of basal cell mitosis which in turn is determined at least in part by the degree of crowding in this layer (Mitrani, 1978).

The epidermal 'collar' seen at the margins of the base of PG's was often a prominent feature, and can be interpreted as an attempt by the epidermis to 'pinch off' the lesion by the process of trans-epidermal elimination. The ability of the epidermis to 'engulf' and extrude abnormal dermal material is well known and has recently been studied experimentally (Bayoumi, Gaskell & Marks, 1978).

Finally, it is interesting to note that in some ways PG's have analogies with psoriasis as both lesions

show a markedly increased epidermopoiesis overlying an extremely active and abnormal dermal papillary vasculature. Further study of the dermo-epidermal relationship in PG's may increase our understanding of the sequence of events leading to the production of the psoriatic plaque.

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REFERENCES

- BAYOUMI, A., GASKELL, S. & MARKS, R. (1978) Development of a model for transepidermal elimination. *British Journal of Dermatology* (in press).
- CHOPRA, D.P. & FLAXMAN, B.A. (1974) Comparative proliferative kinetics of cells from normal human epidermis and benign epidermal hyperplasia (psoriasis) *in vitro*. *Cell and Tissue Kinetics*, **7**, 69.
- CHRISTOPHERS, E. & SCHAUMLOFFEL, E. (1967) Zur DNS-Synthesezeit in der menschlichen Epidermis. *Archiv für klinische und experimentelle Dermatologie*, **228**, 57.
- DAVIES, M.G., ATAI, F. & MARKS, R. (1978) The connective tissue and endothelium in pyogenic granulomata (in preparation).
- DAVIES, M.G. & MARKS, R. (1977) Evolution of the lesion of discoid lupus erythematosus—a comparison with lichen planus. *British Journal of Dermatology*, **97**, 313.
- EVANS, C.D. (1954) Pyogenic granuloma. Multiple local recurrence following excision. *Proceedings of the Royal Society of Medicine*, **47**, 1063.
- FRAIN-BELL, W. (1958) Multiple pyogenic granulomata. *British Journal of Dermatology*, **70**, 428.
- FRANCIS, A.J. & MARKS, R. (1977) Skin stretching and epidermopoiesis. *British Journal of Experimental Pathology*, **58**, 35.
- HEENEN, M. & GALAND, P. (1971) Cell population kinetics in human epidermis: *in vitro* autoradiographic study by double-labelling method. *Journal of Investigative Dermatology*, **56**, 425.
- LACHAPELLE, J.M. (1969) Isotopic labelling of cutaneous structures. *British Journal of Dermatology*, **81**, 299.
- MARKS, R. (1973) Histogenesis of the inflammatory component of rosacea. *Proceedings of the Royal Society of Medicine*, **66**, 742.
- MARKS, R., BLACK, M.M. & WILSON JONES, E. (1973) Epidermal cell kinetics in lichen planus. *British Journal of Dermatology*, **88**, 37.
- MITRANI, E. (1978) Possible role of connective tissue in epidermal neoplasia. *British Journal of Dermatology*, **99**, 233.
- RYAN, T.J. (1978) Personal communication.
- WARNER, J. & WILSON JONES, E. (1968) Pyogenic granuloma recurring with multiple satellites. A report of 11 cases. *British Journal of Dermatology*, **80**, 218.
- WEGENER, K., HOLLWEG, S. & MAURER, W. (1964) Autoradiographische bestimmung der DNS Verdopplungszeit und anderer Teil Phasen der Zell-Zyklus bei Fetalen Zellarten der Ratte. *Zeitschrift für Zellforschung und mikroskopische Anatomie*, **63**, 309.

Towards characterization of epidermal cell migration promotion activity in serum

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SUMMARY

The effect of various fractions of serum on active epidermal cell migration *in vitro* has been studied by incubating small explants of normal human skin in the presence of the fractions and determining the extent of migration after 48 h incubation. Serum was fractionated by combining ammonium sulphate precipitation and molecular sieve chromatography using Sephadex G-200. The components of serum responsible for most, if not all, of the migration promotion activity were localized in the G-200 third peak (albumin fraction) of the supernatant after precipitation of the serum with ammonium sulphate at 50% saturation.

Re-epithelialization plays a vital role in wound healing. Active epidermal cell movement (AECM) is the earliest observed event in re-epithelialization and *in vitro* does not depend on mitotic activity (Nishikawa & Marks, 1973). AECM occurs at the edges of human skin fragments when these are placed in a suitable nutrient medium containing serum. However, in the absence of serum AECM does not occur (Coombs *et al.*, 1974; Marks *et al.*, 1972). We have attempted to isolate the fraction(s) containing the migration promotion activity (MPA) in pooled normal human serum in order to identify the component(s) responsible.

METHODS

Bioassay

Normal human skin was obtained with a Castroviejo keratome set at 0.4 mm from the lateral aspect of the thighs of healthy young male volunteers who had given their informed consent. The thin sheets of skin were divided into rectangular explants approximately 5 × 7 mm. The skin explants were floated epidermis upwards in Eagle's minimal essential medium containing either 10–20% pooled normal human serum (PNHS) or a serum fraction in an equivalent concentration. A volume of 5 ml of the medium and two explants were placed in disposable plastic Petri dishes (sterilin) and incubated

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for 48 h at 37°C in an atmosphere of 95% air and 5% carbon dioxide. After 48 h the explants were fixed in 10% formalin, histologically processed, sectioned and stained with haematoxylin and eosin according to routine methods. Sections at 150 µm intervals along the specimens were examined microscopically and the number of epidermal cells that had migrated at each side of the explant were counted. The results were expressed as the mean number of migrating epidermal cells per site of migration. This technique poses no difficulty for assessment when migration is marked but is less reliable for lesser degrees of migration (Figs 1 and 2). This method is similar to that used by Coombs *et al.* (1974). Each set of experiments included a positive control in which PNHS was added to the medium and a negative control in which nothing was added to the synthetic medium.

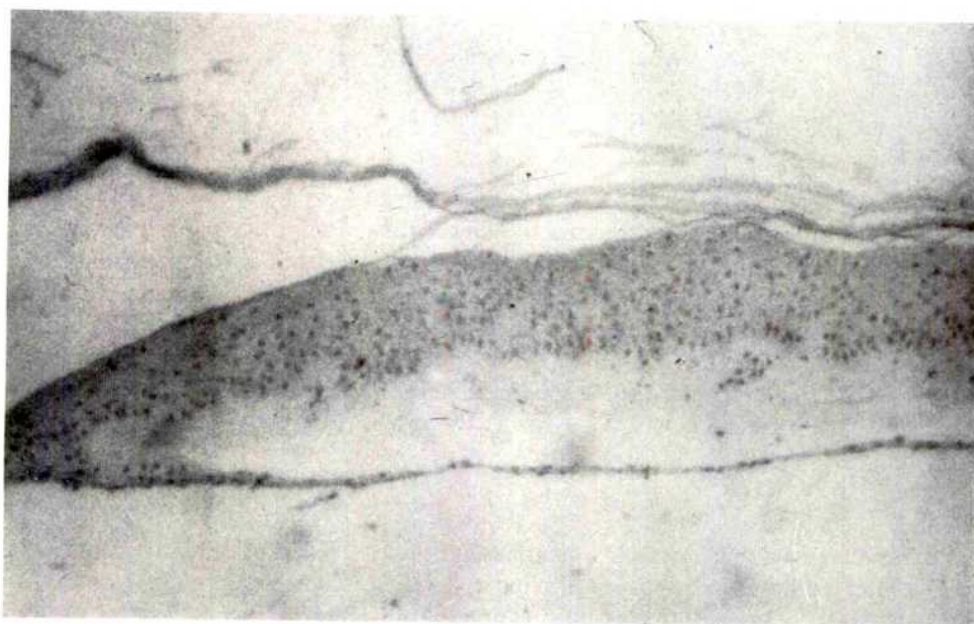


FIGURE 1. Section of human skin after 48 h incubation in the presence of S₅₀C and Eagle's MEM. Long thin migrating epithelial cells are clearly discernible (H & E, × 90).

FRACTIONATION PROCEDURES

Preliminary experiments included water precipitation of the euglobulin fraction of serum. PNHS was dialyzed against distilled deionized water or Coon's Phosphate Buffer* for 24 h at 4°C. The serum after dialysis (dialyzate) was centrifuged for 30 min at 1500 g and the precipitate obtained was collected, freeze dried and stored at -20°C until required. This 'water precipitate' was called Fraction 1. In all subsequent experiments PNHS was preheated at 56°C for 30 min previous to any fractionation procedure.

Protein precipitation with ammonium sulphate was performed at 4°C and at pH 7. The saturated ammonium sulphate solution was applied gradually to the serum through a dialysis membrane over a period of 16 h and the serum fractions obtained after centrifugation were dialyzed extensively against several changes of phosphate buffered saline for a period of 5-7 days at 4°C.

* Coon's phosphate buffered saline: NaCl = 20.25 g, Na₂HPO₄ = 3.20 g, NaH₂PO₄ = 0.39 g, H₂O = 2.50 litres, adjust pH to 7.2.

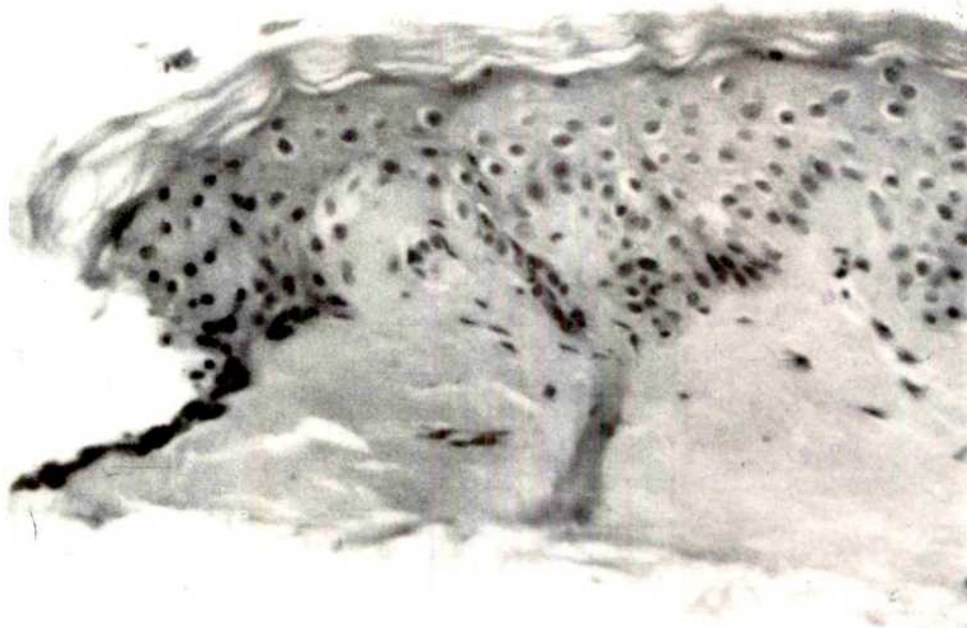


FIGURE 2. Section of human skin after 48 h incubation in the presence of MEM only. The few cells showing migration are not considered significant. (H & E, $\times 90$).

Gel filtration was performed on Sephadex G-200 gel (Pharmacia) in a column of 1000×16 mm. 0.1 M Tris-HCl, 0.5 N NaCl was used as the column buffer at a pH of 8.6 . Fractions pertaining to the same peak were pooled, adjusted to their initial concentration in serum and dialyzed against phosphate buffered saline at 4°C . All fractions were finally sterilized through an Amicon membrane filter of pore size $0.2\ \mu\text{m}$ and stored at -20°C .

RESULTS

Preliminary experiments employed water precipitated material ('F1' fraction) and the results obtained are summarized in Table 1. The apparent loss of activity after dialyzing PNHS against water is recovered in the precipitate (Fraction 1) that is formed in the dialyzate. In fact Fraction 1 was found to possess a considerable degree of MPA and a dose effect relationship was established for this Fraction (Table 2). However, water precipitation is a crude method which often leads to irreproducibility of results.

No clear pattern of MPA emerged when the crude fractions obtained either by ammonium sulphate precipitation or G-200 chromatography were tested. Results at this stage suggested that the serum fractions promoting epidermal migration were contained within the macroglobulin fraction eluted by G-200 chromatography and seemed in agreement with the reports of Ooka *et al.* (1975). Yet, when this fraction was purified further no significant MPA remained in this peak. Serum fractions were then obtained by combining the two above techniques.

Fig. 3 shows the pattern of the supernatant (S50) after serum precipitation with ammonium sulphate to a final concentration of 50% saturation and then submitted to Sephadex G-200 column chromato-

TABLE 1. Preliminary results obtained in experiments using dialysed serum

Addition to medium	Mean no. of migrating cells/site of migration
1. NHS (Normal human serum)	197.0 ± 66.9
2. NHS dialysed in Coon's buffer	126.8 ± 74.2
3. NHS dialysed in H ₂ O	96.8 ± 15.3
4. Preheated PNHS (56° for 30 min)	100.3 ± 43.9
5. Fraction I	99.2 ± 62.7
6. Nothing	0-10

TABLE 2. Dose effect relationship for migration promotion activity with different doses of FI fraction

Addition to medium	Mean no. of migrating cells
32.5 mg of FI	120.33 ± 56.0
16 mg of FI	66.71 ± 25.13
8 mg of FI	46.59 ± 29.78
4 mg of FI	46.08 ± 32.99
2 mg of FI	44.00 ± 11.53
0.5 ml of N. human serum	186.00 ± 39.53

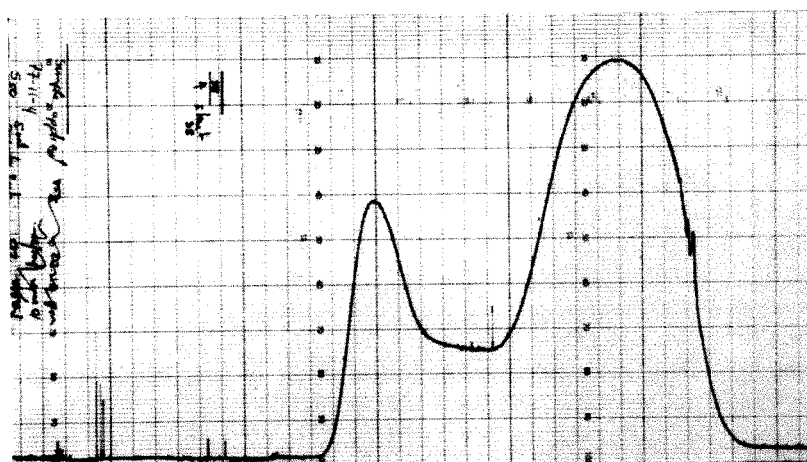


FIGURE 3. Optical density pattern of the supernatant after serum fractionation with 50% saturation ammonium sulphate submitted to sephadex G-200 column chromatography.

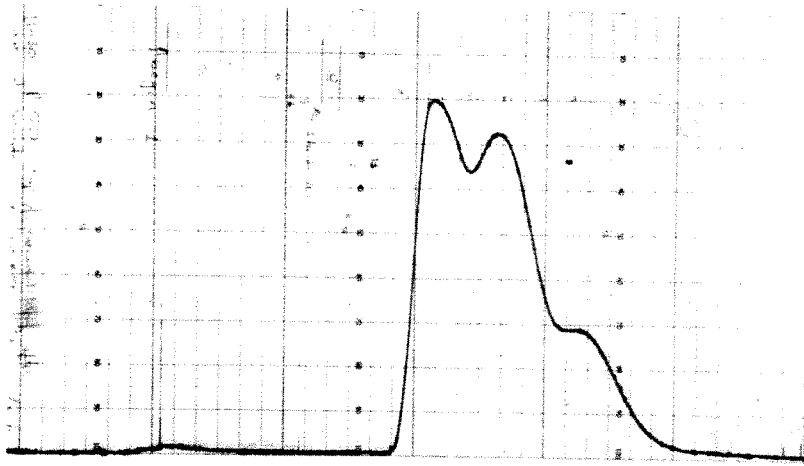


FIGURE 4. Optical density pattern of the precipitate after serum fractionation with 50% saturation ammonium sulphate submitted to sephadex G-200 column chromatography.

graphy. Some of the macroglobulin containing fraction is present in the $S_{50}A$ Fraction. The gamma-globulin containing fraction is almost totally absent, and most of the components of the albumin fraction remain in the supernatant and are contained in Fraction $S_{50}C$. G-200 fractionation of the precipitate of serum after 50% saturation with ammonium sulphate is shown in Fig. 4. This precipitate contains mainly the gamma-globulin fraction ($P_{50}B$) and some of the macroglobulin fraction ($P_{50}A$). Table 3 illustrates the results found when the combined fractions that were obtained after

TABLE 3. Results obtained after combining 50% $(NH_4)_2SO_4$ precipitation and sephadex G-200 column chromatography. Each result is the mean no. of migrating cells per site of migration in 80 sections

Addition to medium	* Volume of fraction per 5 ml medium (ml)	Mean no. of migrating cells per site of migration
$S_{50}A$	0.5	0
	1.0	0
$S_{50}C$	0.5	200
	1.0	200
$P_{50}B$	0.5	40
	1.0	10
$P_{50}A$	0.5	0
	1.0	0
NHS	0.5	200
	1.0	200
Nothing		0-10
		0-10

* The concentration of the added fractions was equivalent to its concentration in whole serum.

precipitation with ammonium sulphate to 50% saturation and fractionation with G-200 were tested for MPA. Each of the results shown is the mean of 8 separate explants and the evaluation of 80 different sections tested with fractions obtained from at least two different pools of normal human serum. The difference in activity between the various fractions is obvious. The components of serum responsible for most if not all of the MPA can be localized in the G-200 third peak of the supernatant after precipitation of PNHS with ammonium sulphate to a final concentration of 50% saturation (S₅₀C).

DISCUSSION

Previously Coombs & Marks (1974) confirmed the migration promotion activity of serum for epidermis in skin explants *in vitro*. These authors believed that the loss of activity observed after dialysis of the serum indicated that the activity resided in a small molecular species. However, the results of the present study indicate that the loss of activity observed was due to the formation of a precipitate in the serum rather than the removal of an active substance by dialysis.

Furthermore, our results do not support the hypothesis put forward by Levine (1972) that the MPA of serum might be indirect. She suggested that alpha-2-macroglobulin inhibits collagenase thus providing a suitable collagen substrate on which epidermal cells can migrate. Alpha-2-macroglobulin is most likely to be contained in the fraction P₅₀A (above) and yet no activity was found within this fraction. The possibility of an indirect effect of serum in promoting migration activity cannot however be totally discarded since alpha-1-antitrypsin is perhaps a constituent of S₅₀C.

It seems unlikely that the MPA effect of serum is related to its tonicity. It is interesting, however, that the epidermis of skin explants is capable of such a degree of migration activity when it is incubated under such restricted conditions in which only a limited number of serum components (S₅₀C) are added to the synthetic medium. Whether we are dealing with a genuine migration promoting factor or a specific tissue requirement is not as yet certain. Both alternatives are equally interesting and may have implications for the treatment of wounds.

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REFERENCES

- COOMBS, V.A., NISSEN, B.K. & MARKS, R. (1974) The epidermal cell migration promotion activity of serum in guinea-pig skin *in vitro*. *Archives für Dermatologische Forschung*, **249**, 367.
- LEVINE, M. (1972) The growth of adult human skin *in vitro*. *British Journal of Dermatology*, **86**, 481.
- MARKS, R., BHOGAL, B. & DAWBER, R.P.R. (1972) The migratory property of epidermis *in vitro*. *Archives für Dermatologische Forschung*, **243**, 209.
- NISHIKAWA, T. & MARKS, R. (1973) Active epidermal cell movement in human skin *in vitro*. *British Journal of Dermatology*, **88**, 215.
- OOKA, H., YAMAMOTO, K., OKUMA, Y., SUGA, S. & WAKASUGI, M. (1975) The migratory activity of rat epidermal cells *in vitro*—age related changes and the effect of serum. *Experimental Gerontology*, **10**, 79.

A new form of cellular arrangement in guinea-pig ear epidermis

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SUMMARY

A new type of squame arrangement is described in guinea-pig ear epidermis. It is characterized by overlapping obliquely-angled outer cells and squames, which thus form a roof-tile pattern. This pattern develops in epidermis that is thicker than about 42 μm ; in thinner epidermis the outer cells and squames form the usual columnar stacks. It is concluded that the roof-tile pattern, which is usually directed towards the ear edge, is typical of slight to moderate hyperplasia. In extreme hyperplasia, when the strata granulosum and corneum are destroyed, only cellular disorder is seen.

In 1969 Mackenzie first reported a form of epidermal cellular architecture in which the keratinizing distal cells and the keratinized squames are arranged in columns. Since then the same type of arrangement has been found in mouse, hamster and guinea-pig, as well as in certain regions of human epidermis, and the phenomenon has been extensively studied (Mackenzie, 1970; Christophers, 1971a, b; Allen & Potten, 1976). There has also been much speculation on the mechanism by which this cellular pattern is achieved (Potten & Allen, 1975).

So far the only variation from this pattern that has been found is its complete breakdown in hyperplasia. In this condition the new cells, which are produced faster and also move faster to their death at the base of the stratum corneum (Bullough, 1975), seem simply to move outwards in a disordered mass (Christophers, 1972).

Now, however, during a study of the organisation of guinea-pig ear epidermis, another pattern of epidermal cell arrangement has been detected and this is described below.

MATERIAL AND METHODS

The 17 animals used were all adult male guinea-pigs purchased commercially. Many of the ears showed signs of old damage with healed tears and even, in a few cases, with small pieces missing altogether. Before being used in the experiments each guinea-pig was caged separately for about 3 weeks partly to acclimatize it and partly to reduce the risk of any further damage to its ears.

In each animal one ear was kept as an untreated control while the other was wetted daily for 8 days with a mixture of 7 parts n-hexadecane and 3 parts acetone.

After death small pieces of ear, taken from the ear edge, were fixed in Bouin's fluid. Each ear sample was then sectioned at right angle to the ear edge, which is a necessary procedure to reveal fully the cellular organization described below. Sections were cut at 7 μ m and stained in Ehrlich's haematoxylin and eosin.

OBSERVATIONS

The control epidermis

In the untreated epidermis it was immediately obvious on the outer surface of the stratum granulosum that the cells formed an overlapping pattern like the tiles of a roof (Fig. 1 b, c). In the sections each superficial granulosum cell had attached to it a chain of squames that ran diagonally outwards to the surface of the stratum corneum (Fig. 1b, 2a). Thus the stratum corneum must have been composed of sheets of squames, which instead of lying parallel to the epidermal surface were set at an angle to that surface.

Furthermore, instead of each squame being set directly one upon another, as in the stacked-cell columnar pattern, it was set above the junction of the two or more squames beneath it (Fig. 2a). Thus, while the cells in the common stacked-cell pattern form vertical columns, those in the roof-tile pattern form diagonal rows. Where the structure of the stratum corneum had been loosened by the passage through it of the microtome knife, it was evident that the strongest bonds between adjacent squames were not only in their edge-to-edge junctions, as in the columnar pattern, but also in the connexions between these junctions and the contiguous regions of the squames below but not of those above (Fig. 2a).

The surface of the stratum corneum was then examined with a stereoscan electron microscope, and in favourable regions, the roof-tile pattern of squame arrangement that was to be expected from the evidence of the sections was confirmed (Fig. 2b).

The inclination of the squame sheets was next considered and it was found that, both on the inner and outer ear surfaces, this was usually directed in such a way that the free squame edges on the corneum surface pointed towards the ear edges. This was true of 15 of the 17 ears examined; in the other 2 ears the squames of one surface were directed towards, while those of the other surface were directed away from, the ear edge. No instances were found where the squames of both surfaces were directed away from the ear edge.

In all cases the roof-tile arrangement of the squames ceased close to the edge of the ear. In this edge region the cells of the stratum granulosum and the overlying squames lay parallel to the epidermal surface. However, these cells and squames did not stack into columns; they remained staggered with the centre of each cell or squame set above the junction of the two or more cells or squames below.

The arrangement of the epidermal cells

As already explained, the cells of the superficial stratum granulosum were angled to the surface, each being joined edgewise to a squame outwards and to a flattened stratum spinosum cell inwards. Indeed the diagonal pattern and alternating arrangement of the squames was prefaced by the diagonal pattern and alternating arrangement of 2 or 3 layers of outer epidermal cells, both of the stratum granulosum and of the superficial stratum spinosum (Fig. 1b).

Beneath these flattened and flattening cells, in the mid-epidermal region, the cells were roughly cubical, while in the lowest epidermal layer they were columnar. These facts were more readily apparent from the shapes of the nuclei than from the cell outlines, which were not always easily distinguishable. The impression given was that, in order to fit into the diagonal pattern at the surface,

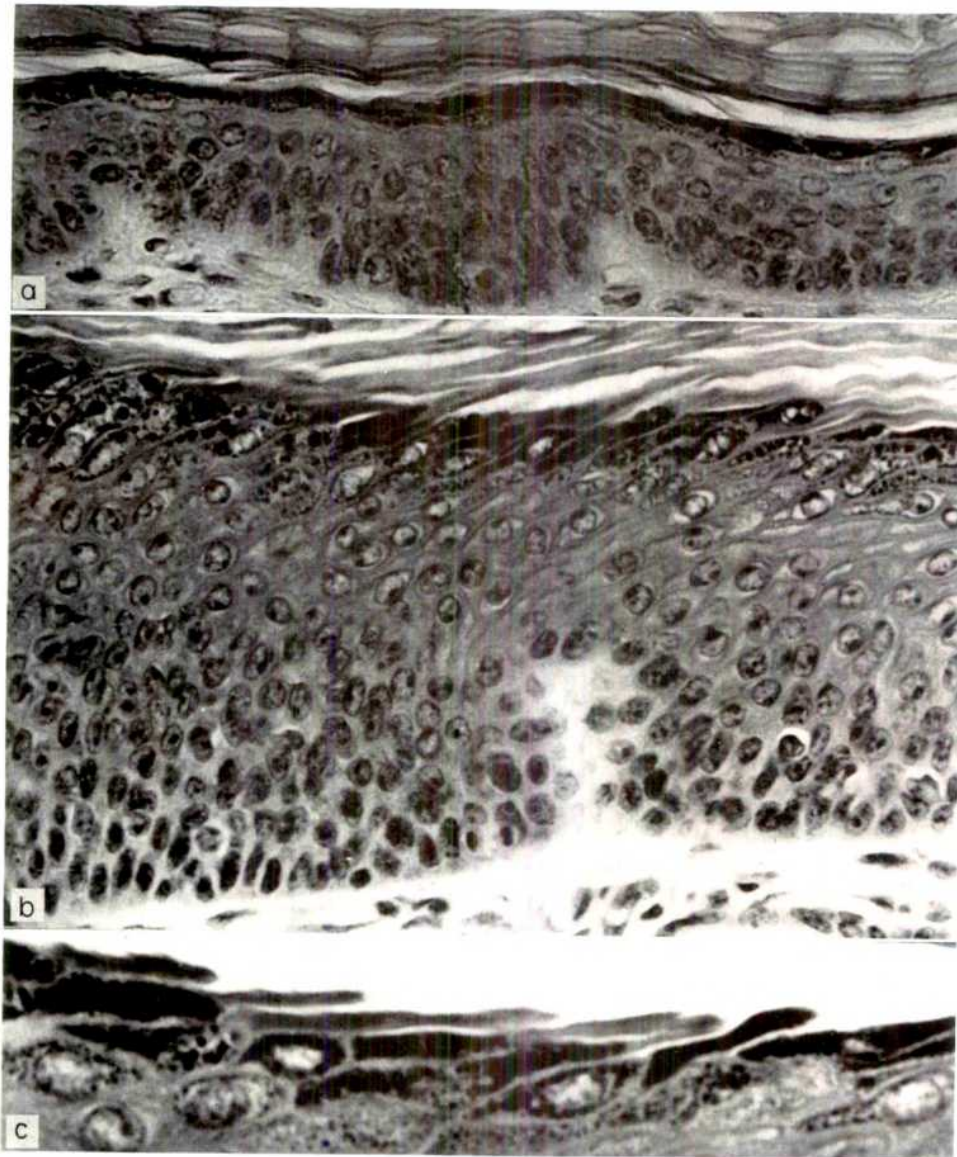


FIGURE 1. (a) Thin epidermis of guinea-pig ear showing the columnar stacking of the squames ($\times 540$). (b) Thick epidermis of guinea-pig ear showing the roof-tile arrangement of the distal cells and squames ($\times 540$). (c) Stratum granulosum of thick guinea-pig ear epidermis showing the roof-tile arrangement of the cells ($\times 825$).

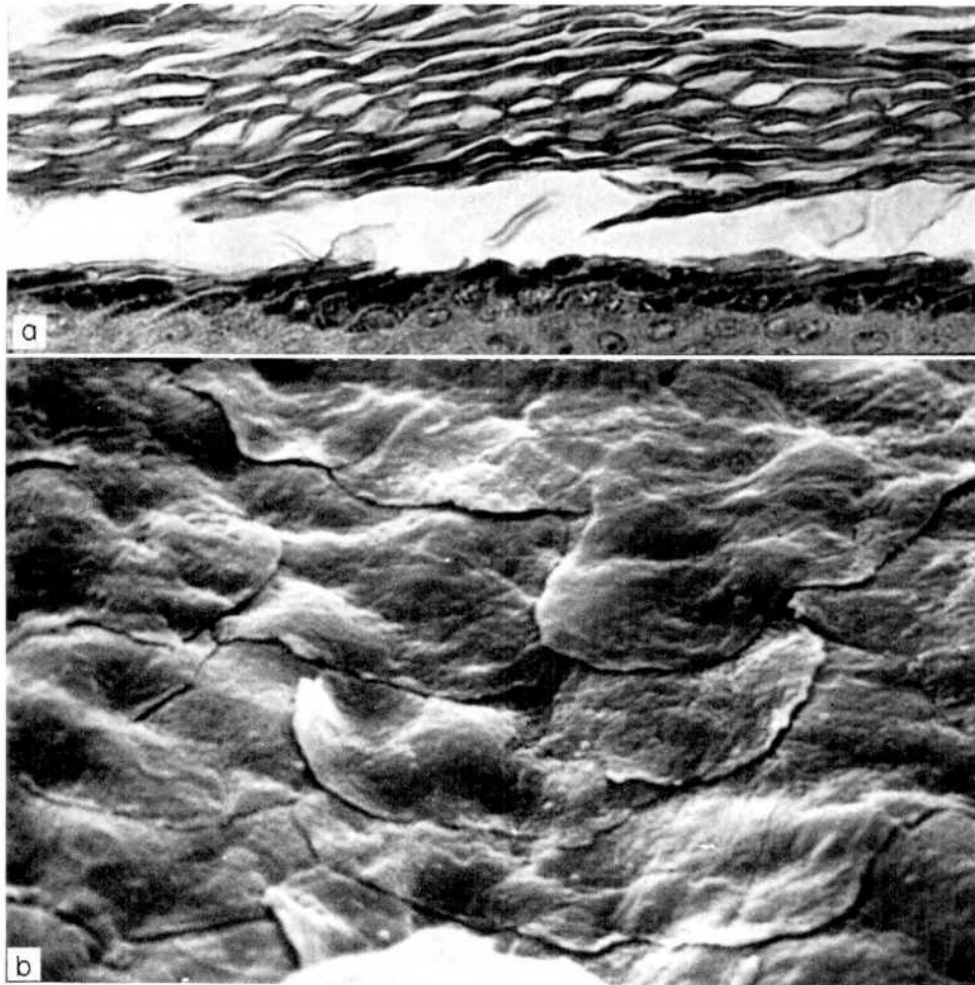


FIGURE 2. (a) Section of stratum corneum showing the inclined sheets of squames leading from the baseline to the surface ($\times 720$). (b) Stereoscan photograph showing the roof-tile arrangement of squames on the surface of the stratum corneum ($\times 2500$).

the rising cells describe an arc, at first passing straight up and then inclining more and more as they flatten and slide sideways towards the surface (Fig. 1b). However, it is probable that the cells in fact rise vertically, fitting into the overlapping and diagonal pattern as they go.

The effect of epidermal thickness

Christophers (1972) has described how the structural pattern of guinea-pig ear epidermis changes with the epidermal thickness, which itself changes with the mitotic rate. In thinner epidermis the columnar pattern of stacked cells is seen; when the epidermis thickens this pattern changes to one of cellular disorder. He stated that the change from 'order to disorder occurs at an average thickness of 38–46 μm above which no cell columns are found', and he added that normal guinea-pig ear epidermis is so close to this critical thickness that it 'is normally at the margin between order and disorder'.

The epidermal thickness in the untreated ears used in the present study was therefore measured and was found to be mostly greater than the critical 38–46 μm range. In only 3 out of 17 animals was it thinner on average than 46 μm . In these 3 animals the thinner parts of the epidermis showed the typical stacked columns (Fig. 1a) described by Christophers (1972), while the thicker parts showed the roof-tile pattern. In the other 14 animals the average epidermal thickness was between 46 and 73 μm and all showed the roof-tile pattern.

It is therefore clear that the roof-tile pattern described here relates only to epidermis that is thicker than the critical range and thus to the condition described by Christophers as disordered. This discrepancy of observation is perhaps not surprising since, if the sections are not cut at right angles to the ear edge, the roof-tile pattern is not easily seen. Conversely, in thinner epidermis, the stacked columns of cells are seen in sections cut at any angle.

The effects of hyperplasia

While any guinea-pig ear epidermis thicker than the critical range may perhaps be regarded as in some degree hyperplastic, due for instance to scratching, it is important to determine what happens to the roof-tile cell pattern in more extreme hyperplasia. In the ears here treated with n-hexadecane the average epidermal thickness ranged between 96 and 165 μm .

In examining this epidermis it proved necessary to distinguish between areas in which the stratum granulosum and at least part of the overlying stratum corneum remained in existence and areas in which these layers had been largely or wholly destroyed. In the former areas the roof-tile pattern persisted; in the latter areas, which were usually covered by an exudate, there was indeed cellular disorder.

Where the roof-tile pattern did persist, it was nowhere so obvious as in the untreated ears. This was because the cells of the stratum granulosum were not so strongly angled to the epidermal surface. However, with a thicker stratum granulosum, the alternating cell pattern, in which the centre of one granulosum cell lay above the junction of the two or more cells below, was particularly clear, and careful examination confirmed that the diagonally arranged sheets of outer cells and of squames were still present.

The direction of inclination of these sheets, towards or away from the ear edge, was also determined. In 10 of the 17 animals both the outer and inner cell and squame sheets were inclined towards the ear edge; in 1 animal the inclination was on one side towards and on the other side away from the ear edge; in 4 animals the inclination on one side was towards the ear edge while the situation on the other side could not be determined because of heavy damage; and in the remaining 2 animals the damage on both sides was so extensive that no conclusion could be reached. However, even in heavily damaged epidermis there were sometimes small patches in which both the strata granulosum and corneum remained, and in these, except in one case of reversed inclination, the sheets were inclined towards the ear edge.

DISCUSSION

Previously published evidence on mammalian epidermal structure has led to the common belief that, at least below a certain mitotic rate, the columnar stacking of the distal cells and squames is the norm; with a higher mitotic rate only cellular disorder is to be seen. In guinea-pig ear it is now clear that cellular disorder is only to be found in extreme forms of hyperplasia; in moderate hyperplasia the distal cells form a roof-tile pattern. Whether this pattern is also to be found in other types of epidermis, normal or hyperplastic, is not yet known, but preliminary searches in mouse hyperplastic ear epidermis and in psoriasis have not revealed it.

The main problems now arising concern the mechanisms by which such patterns are produced and, in the guinea-pig ear, the reason for the sudden switch from one pattern to the other whenever the epidermal thickness (i.e. the mitotic rate) varies across a critical point.

All types of epidermis share a common basic structure: the basal cell layer is fixed firmly to the dermal surface (Bullough & Deol, 1975; Bullough & Mitrani, 1976); the intermediate cell zone is relatively fluid in that the cells can move laterally as well as distally in order to reach their appropriate places in the overlying cellular pattern; the distal zone is a rigid region in which the cells are glued so tightly together and to the overlying squames that they can no longer move independently. Thus the problem of pattern formation is the problem of what controls the movements of cells that have left the rigid basal layer and not yet entered the rigid distal zone.

In guinea-pig ear the cell patterns produced in the distal zone are clearly related to the width of the intermediate zone: if this is narrow (c. 2 cell layers) the result is cell stacking into columns; if it is thicker (>c. 3 cell layers) the result is the roof-tile pattern; if it is very thick and badly damaged the result is cellular disorder.

In the stacked cell type of epidermis the mitotic rate is relatively low and the cells that are forced out of the basal layer move relatively slowly across the narrow intermediate zone. In the distal zone the laterally adjacent cells and squames of neighbouring stacks are alternately placed, one being slightly higher than its neighbour (see Christophers, Wolff & Laurence, 1974). In Fitzgerald's words (1977) 'the squames from adjacent stacks are perfectly riffle-shuffled, their edges alternating with mathematical precision'. It follows that the inner surface of the distal zone cannot be flat; at the base of every alternate stack there must be a slight depression into which the next cell to enter the pattern may fit itself. In other words the inner surface of the pattern may act as a template to maintain the ordered structure. The complexities of this system have been well analysed by Potten & Allen (1975).

The original creation of the cell stacks, and their recreation after their destruction in hyperplasia, may then depend on the tendency of the keratinizing cells to fuse not edge to edge but in a staggered manner, the edge of the first cell to be formed overriding the edges of the adjacent cells that are formed slightly later. As suggested by Allen & Potten (1976) this could occur naturally if the squames have a particular form of a flattened tetrakaidecahedron (14-sided figure).

However, in guinea-pig ear, when the mitotic rate rises and the epidermis thickens, the greater numbers of new cells cause the intermediate zone to widen and the distal cell flow to accelerate (Bullough, 1975). This appears to result in the greater crowding of the distal cells of the stratum spinosum as they expand and flatten. In theory, the pressure so caused could be relieved if these cells simply entered the bases of the cells stacks more quickly; in practice, the pressure appears to be relieved in two successive stages. In the first stage, the distal stratum spinosum cells are fitted together more closely by setting the thicker central regions of the cells (where the nucleus still persists) against the thinner peripheral regions of the cells above, that is by overlapping the cells (as with the overlapping packing of bubbles: see Mackenzie, 1972); in the second stage, with still tighter packing, the flattening cells become obliquely angled to form the roof-tile pattern. Once this pattern has been formed its base may also act as a template guiding the next layer of cells into their appropriate overlapping and slanting positions.

Support for this two-stage response to increasing pressure comes from two observations. The first relates to ears showing both cell stacking in the thinner epidermis and the roof-tile pattern in the thicker epidermis; the transition zones between these regions show overlapping but not slanting cells. The second comes from the epidermis of the ear edge. In this region, because of the curve of the edge, a narrower area of basal cell production is overlain by a wider area of distal cell keratinization. The cell crowding in the distal layer should therefore be less than it is elsewhere and it is in this region that cell overlapping without the roof-tile pattern is always seen.

The manner of control of new cell production in epidermis and in other tissues has been extensively studied in recent years (see Bullough, 1975). The manner in which the newly-formed tissue cells then move into position to create and maintain the typical tissue architecture is a new field of study now opening. The manner in which old cells die and are disposed of is the third aspect of this problem of tissue homeostasis and is also a relatively unexplored field.

REFERENCES

- ALLEN, T.D. & POTTEN, C.S. (1976) Significance of cell shape in tissue architecture. *Nature*, **264**, 545.
- BULLOUGH, W.S. (1975) Mitotic control in adult mammalian tissues. *Biological Reviews*, **50**, 99.
- BULLOUGH, W.S. & DEOL, J.U.R. (1975) Dermo-epidermal adhesion and its effect on epidermal structure in the mouse. *British Journal of Dermatology*, **93**, 417.
- BULLOUGH, W.S. & MITRANI, E. (1976) An analysis of the epidermal control mechanism. In: *Chalones* (Ed. J.C. Houck), chpt. 2. North-Holland Publishing Company, New York.
- CHRISTOPHERS, E. (1971a) Cellular architecture of the stratum corneum. *Journal of Investigative Dermatology*, **56**, 165.
- CHRISTOPHERS, E. (1971b) Die epidermale Columnärstruktur. *Zeitschrift für Zellforschung*, **114**, 441.
- CHRISTOPHERS, E. (1972) Correlation between column formation, thickness and rate of new cell production in guinea-pig epidermis. *Virchows Archiv, Abteilung B, Zellpathologie*, **10**, 286.
- CHRISTOPHERS, E., WOLFF, H.W. & LAURENCE, E.B. (1974) The formation of epidermal cell columns. *Journal of Investigative Dermatology*, **62**, 555.
- FITZGERALD, M.J.T. (1977) New knowledge about the epidermis. *Irish Journal of Medical Science*, **146**, 206.
- MACKENZIE, I.C. (1969) Ordered structure of the stratum corneum of mammalian skin. *Nature*, **222**, 881.
- MACKENZIE, I.C. (1970) Relation between mitosis and the ordered structure of the stratum corneum in mouse epidermis. *Nature*, **226**, 653.
- MACKENZIE, I.C. (1972) The ordered structure of mammalian epidermis. In: *Epidermal Wound Healing* (Ed. H.I. Maibach and D.T. Rovee), chpt. 1. Year Book Medical Publishers, Chicago.
- POTTEN, C.S. & ALLEN, T.D. (1975) Control of epidermal proliferative units. *Differentiation*, **3**, 161.

Human infections with *Microsporum gypseum* in Denmark

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SUMMARY

Ninety cases of *Microsporum gypseum* infection in Denmark during the period 1933–1977 are reviewed. The sex ratio, age distribution, and anatomical sites are stated. A case of onychomycosis in a 1-year-old child is reported, and the cases of *M. gypseum* onychomycosis in the literature are reviewed.

C. Rasch was the first in Denmark to publish details of dermatomycoses caused by *Microsporum gypseum* (Bodin) Guiart & Grigorakis 1928 (*M. gypseum*), a total of 4 cases (Rasch, 1922). However, three cases of similar infections had already been diagnosed by Bang (Bang, unpublished, cf. Sylvest, 1949). Later, S. Lomholt (1933) reported a case of *M. gypseum* infection, and in 1949 Sylvest analysed the incidence of this fungus in Denmark during the period 1933–1947, which amounted to 12 cases. Since that time only a single short report on *M. gypseum* infection in Denmark has appeared (Haxthausen, 1950).

The present study comprises all cases diagnosed in the Finsen Institute from 1933 up to the present day, including Sylvest's 12 patients, a total of 90.

SUBJECTS AND METHODS

The material consists of samples from the patients seen in the Dermatological Department of the Finsen Institute, Copenhagen, from 1933 until this day (59 patients) and, from 1960, samples sent to our Mycological Laboratory from doctors all over the country (31 patients)—a total of 90 cases.

The method of the mycological study has been (1) examination of skin scrapings, hair and nail fragments by direct microscopy following treatment in a solution of KOH and (2) incubation of the material on Sabouraud's glucose-peptone agar to which had been added various antibiotics for inhibiting bacteria and mould fungi.

RESULTS

M. gypseum was isolated by culture from 90 patients. In all cases the site of the mycosis is known and

so is the result of direct microscopy of which 94% had been positive. In 89 cases the patient's sex (Table 1) and date of first consultation (Table 3) are known, but the age is known for only 82 patients (Table 1).

TABLE 1. Sex and age of 82 patients with *Microsporum gypsum* infection in Denmark (1933-1977)

Age	Males	Females	Patients
0-14	41%	59%	37 (100%)
15+	11%	89%	45 (100%)

Of the 90 patients four were infected in more than one region, two on the face and on the trunk, one on the face and on the neck, and one on the trunk, arm, and leg, while in the remainder only one region was involved. The distribution is given in Table 2.

TABLE 2. Regional distribution of *Microsporum gypsum* infection in 86 patients from Denmark (1933-1977)

Region/age	0-14	15+	?	Patients	%
Face and neck	11	20	3	34	39.6
Arm and hand	7	14	2	23	26.7
Scalp	5		2	7	8.1
Chest and back	4	3		7	8.1
Buttocks and thighs	6	1		7	8.1
Foot and lower leg		5		5	5.9
Nails	1	2		3	3.5
Total	34	45	7	86	100.1

From 3 patients *M. gypsum* was isolated from the nails. One of these isolates was from toe nails, with positive direct microscopy, but on culture there proved to be associated growth of *Trichophyton mentagrophytes* var. *interdigitale*. The other two isolates were from finger nails, one from a 67-year-old man in whom direct microscopy had been negative and the other from a 1-year-old boy. In the latter case two investigations were performed at an interval of 2 months. Both samples showed numerous fungal elements on direct microscopy, and on culture pure cultures of *M. gypsum* were obtained,

TABLE 3. Monthly distribution of sampling from patients with *Microsporum gypsum* infection

	1	2	3	4	5	6	7	8	9	10	11	12	Total
Denmark (1933-1977)	17	10	5	6	7	9	5	13	6	6	3	2	89
Czechoslovakia* (1945-1968)	2	0	1	1	3	1	3	7	6	8	7	3	42

* Chmel & Buchvald (1970).

with 22 and 14 colonies respectively. Of the 7 patients in whom the scalp was involved two had kerions: a 2-year-old girl and a 5-year-old boy. All the remaining 80 patients had typical tinea circinata, regardless of the localization.

Among the 90 patients familial occurrence was observed only twice—once in twin boys aged 5 and once in a woman and her niece.

As the first 79 patients were analysed in retrospect, only the cultural habit and the microscopic examination of the isolates had been investigated in order to classify the fungi taxonomically. Isolates from the last 11 patients, i.e. from 1974 until now, were tested by mating experiments; all were found to belong to *Nannizzia gypsea* (Nannizzi) Stockdale, 1963.

DISCUSSION

Many investigations have revealed that *M. gypseum* is a soil-inhabiting dermatophyte of cosmopolitan spread (Ajello, 1953; Pålsson, 1968; Schönborn, 1967).

On the other hand, human *M. gypseum* infections are rarely reported. Ajello (1953), analysing all cases reported up to 1953, found a world total of 270, and among 56,258 cases of dermatomycosis in East Germany between 1955 and 1971 *M. gypseum* made up only 64 or about 0.1% (Blaschke-Hellmessen, 1975).

Microsporum gypseum has been isolated not only from man but also from a large number of animal species having clinical dermatomycoses (Ainsworth & Austwick, 1973).

Clinically a *M. gypseum* infection generally manifests itself as tinea circinata on un-haired, unprotected parts of the body, such as the arms, legs, hands, neck, and especially the face. Of our 90 patients, 74.4% had these sites involved. A similar distribution has been reported by others (Chmel & Buchvald, 1970; Blaschke-Hellmessen, 1976).

Infection of the scalp may occur with or without kerion formation. The present material includes 2 such cases, and Rasch (1922) described two, in boys aged 5 and 9.

Tinea on the body and especially tinea pedis are rarely present in *M. gypseum* infection, in the present material in only 1 patient. Tinea cruris did not occur in our material, but a few cases are on record (Chmel & Buchvald, 1970; Cawley & Grekin, 1949).

Onychomycosis or tinea unguium due to *M. gypseum* is extremely rare. In the present material it was demonstrated with certainty only once. From Table 4 it is apparent that tinea unguium is most common in women over 20 years of age.

In 43 patients, whose age distribution they stated, Chmel & Buchvald (1970) found 52.4% to be from 0 to 14 years; this age group was affected in 44.3% of the present material.

TABLE 4. Cases of *Microsporum gypseum* onychomycosis on record

Author	Year	No. of patients		
Ruiz	1953	1	?	♀, Finger nails
Fegeler	1955	1	24	♀, Finger nails
Rosenthal <i>et al.</i>	1958	1	34	♀, Finger nails
Herpay & Pintye	1965	1	39	♀, Toe nails
Böhme	1965	1	20	♀, Finger nails
Saulov	1965	1	6	♀, Finger nails
Herpay & Rieth	1966	1	39	♀,
Blaschke-Hellmessen	1975	?		?

The monthly incidence of *M. gypsum* infections has been investigated by Chmel & Buchvald (1970) in Czechoslovakia and as may be seen from Table 3 a similar distribution could not be demonstrated in the Danish material, which indeed also showed differences from Chmel & Buchvald's as regards the sex ratio and age distribution; but it should be pointed out that life styles and climates in the two countries are sufficiently different to result in behavioural differences which would affect exposure to this geophilic fungus.

M. gypsum infections are so rarely reported that it is difficult to collect enough material to give an overall impression of this fungus as a cause of dermatomycosis.

REFERENCES

- AINSWORTH, G.C. & AUSTWICK, P.K.C. (1973) *Fungus Diseases of Animals*, 2nd edn. Commonwealth Agricultural Bureaux, Slough, England.
- AJELLO, L. (1953) The dermatophyte, *Microsporum gypsum*, as a saprophyte and parasite. *Journal of Investigative Dermatology*, **21**, 157.
- BANG, H. *Favus, Trichophyti, Microspori* (Manuscript).
- BLASCHKE-HELLMESSEN, R. (1975) Statistischer Bericht über die Dermatophytenflora bei Dermatomykosen in der DDR von 1967 bis 1971. *Dermatologische Monatsschrift*, **161**, 433.
- BÖHME, H. (1965) Vorkommen von *Microsporum gypsum* als Erreger von Mykosen und als Boden saprophyt in Berlin und seiner Umgebung. *Dermatologische Monatsschrift*, **151**, 1279.
- CAWLEY, E.P. & GREKIN, R.H. (1949) Parafavus restricted to the scrotum. *Archives of Dermatology and Syphilology*, **60**, 435.
- CHMEL, L. & BUCHVALD, J. (1970) Ecology and transmission of *Microsporum gypsum* from soil to man. *Sabouraudia*, **8**, 149.
- FEGELER, F. (1955) Mykosen durch *Microsporum gypsum*. *Der Hautarzt*, **6**, 183.
- HAXTHAUSEN, H. (1950) Treatment of superficial trichophytosis with CO₂-snow. *Acta dermato-venereologica*, **30**, 405.
- HERPAY, Z. & PINTYE, I. (1965) Onychomykose und andere Dermatomykosen durch *Microsporum gypsum* in Ungarn. *Mykosen*, **8**, 177.
- HERPAY, Z. & RIETH, H. (1966) Nagelmikrosporidie durch *Microsporum gypsum*. *Bulletin of Pharmaceutical Research Institute*, **65**, 11.
- LOMHOLT, S. (1933) Eczema of the feet caused by the Epidermophyton interdigitale. *Acta dermato-venereologica*, **13**, 432.
- PALSSON, G. (1968) Geophilic dermatophytes in the soil in Sweden. Studies on their occurrence and pathogenic properties. *Acta Veterinaria Scandinavica*, Suppl. **25**.
- RASCH, C. (1922) Skin affections caused by *Achorion gypsum* (Bodin). *The British Journal of Dermatology and Syphilis*, **34**, 1.
- ROSENTHAL, S.A., FURNARI, D. & TOLMACH, J.A. (1958) Onychomycosis due to *Microsporum gypsum*. *Archives of Dermatology and Syphilology*, **78**, 759.
- RUIZ, A. (1953) Un caso de tinea unguinum producido por *Microsporum gypsum* (Bodin, 1907) Guiart et Grigorakis, 1928. *Revista de Biología Tropical*, **1**, 33.
- SAULOV, I. (1965) Die Nagelpilzformen im Gebiet von Plovdiv/Südbulgarien. *Dermatologische Wochenschrift*, **151**, 716.
- SCHÖNBORN, C. (1967) Vergleichende Untersuchungen an geophilen *Microsporum*-Stämmen edaphischer, animaler und humaner Herkunft (1. und 2. Teil). *Mykosen*, **10**, 283 und 425.
- SYLVEST, B. (1949) The incidence of dermatophytes in Denmark. *Acta dermato-venereologica*, **29**, 225.

Human infections with *Microsporum persicolor* in Denmark

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SUMMARY

The geographical and ecological spread of *Microsporum persicolor* is reviewed. It is concluded that *M. persicolor* is a zoophilic dermatophyte prevalent mainly in Europe and seldom reported as the cause of human infection. Twelve Danish cases of *M. persicolor* infection are described—their clinical features, mycological findings, and treatment. The localization of these fungal infections is reviewed on the basis of the present material and 58 cases from the literature.

Microsporum persicolor (Sabouraud) Guiart et Grigorakis, 1928, was originally classified as *Trichophyton persicolor* by Sabouraud who had found it in two cases of human dermatomycosis (Sabouraud, 1910). Since then, this dermatophyte had been isolated from dermatomycoses in most European countries and in a few countries overseas (Table 1).

Microsporum persicolor has been collected not only from human mycoses, but also from various mammals, both with and without clinical manifestations. Most often the fungus has been found in the bank vole, and field vole (English, 1967; English, 1968; English & Southern, 1967; Houin *et al.*, 1972; Mariat, Chatelain & Rouffand, 1975), but also in wood mice (English, 1967; Houin *et al.*, 1972), the common shrew (English, 1967), pipistrelle bat (English, 1966), horse (Schönborn *et al.*, 1971), and dog (Pätiälä, 1945).

Microsporum persicolor has also been isolated from the soil in Bulgaria, India and South Africa (Padhye & Ajello, 1974).

SUBJECTS AND RESULTS

The present report deals with 12 human cases of *M. persicolor* infection in Denmark one of which (L 26046) has been reported previously (Sylvest, 1949).

CLINICAL FINDINGS

The clinical findings are listed in Table 2.

TABLE 1. Geographic distribution of human *Microsporium persicolor* infections

Country	Patients	References
<u>Europe</u>		
Austria	1	Alexander (1922)
Belgium	3	Stockdale (1953), Vanbreuseghem (1949)
Bulgaria	1	Collinge & Stockdale (1973)
Denmark	1	Sylvest (1949)
East Germany	15	Blaschke-Hellmessen (1975), Bruhns & Alexander (1928), Ueckert (1959)
England	19	Duncan (1945), English (1966), Stockdale (1953), Walker (1950)
France	6	Doby <i>et al.</i> (1962), Doby-Dubois <i>et al.</i> (1961), Sabouraud (1910), Stockdale (1953)
Italy	1	Spada & Zonchello (1952)
Yugoslavia	4	Milochevitch (1934), Stockdale (1967)
Switzerland	11	Grigoriu & Brot (1975), Wissel (1956)
West Germany	62	Karrenberg (1928), Keller (1926), Rieth (1956)
<u>Outside Europe</u>		
Algiers	1	Montpellier & Matamoros (1927)
Canada	6	Blank (1955), Blank & Ereaux (1964)
Israel	1	Sagher (1947)
United States	3	Padhye <i>et al.</i> (1973)
Uruguay	2	Conti-Diaz (1964)

TABLE 2. Clinical data for 12 diagnosed cases of *Microsporium persicolor* infections in Denmark

Year No.	Age	Sex	Site	Clinical manifestation	Treatment
1945 L 26046	15	F	Lower leg	Tinea circinata	Sulphur anthrarobin paste
1960 L 72908	52	F	Forearm	Tinea circinata	Griseofulvin
1965 S 6320	33	F	Foot	Tinea pedis	Griseofulvin
1965 S 6740	31	F	Sole	Tinea circinata with id reaction	0.1% potassium permanganate
1967 S 14305	12	F	Foot	Tinea pedis	Griseofulvin
1968 S 18419	57	F	Foot	Eczematous rash	?
1968 S 18576	44	F	Lower leg	Eczematous rash	Griseofulvin
1971 F 45	5	M	Arch of foot	Tinea circinata	Tolnaftate
1975 S 70067	55	M	Hand (wrist)	Tinea circinata with Kerion	Griseofulvin
1975 S 70638	68	F	Hand	Herpes-like with cutaneous necrosis and subcut. foci of pus	Clotrimazole
1976 S 80855	42	M	Forearm	Tinea circinata	?
1977 S 93129	54	F	Hand	Tinea circinata	Clotrimazole

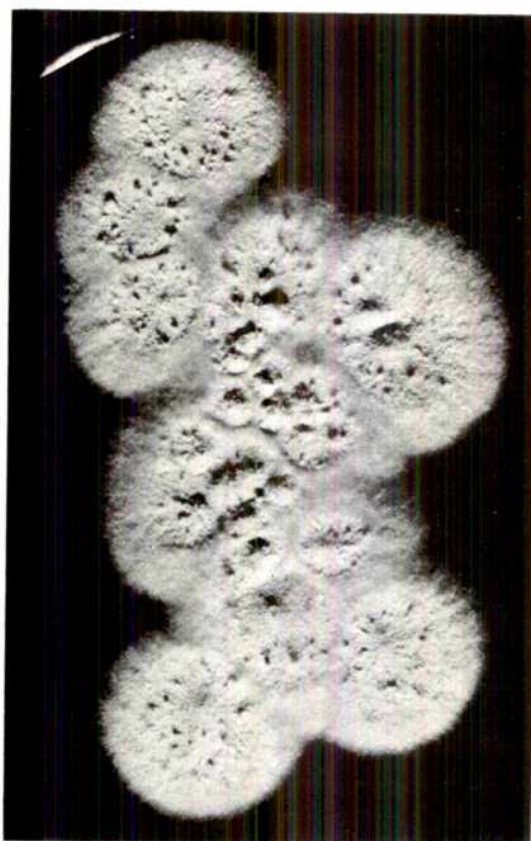


FIGURE 1. Primary isolate of *Microsporum persicolor* after 10 days at $23 \pm 1^\circ\text{C}$ on Sabouraud's glucose peptone agar with chloramphenicol and cycloheximide.

Mycological studies

Direct microscopy. Scrapings from the periphery of the affected areas were studied in KOH preparations. In 11 patients these showed typical dermatophyte structure, consisting of hyphae and arthrosporic chains, while in one case the microscopy was negative.

Appearance of colony. On Sabouraud's glucose-peptone agar to which had been added chloramphenicol 40 mg/l and cycloheximide 500 mg/l, *M. persicolor* grew at room temperature, $23 \pm 1^\circ\text{C}$, at a rate of 15 mm/6 days. In colour it was centrally white, with a wide pink to reddish-purple zone, and peripherally again a narrow white marginal zone. The under aspect was yellowish brown with a darker centre. The surface was slightly rough to woolly (Fig. 1). On Littman's ox bile agar the growth rate was 11 mm/6 days and on malt agar 17 mm/6 days. On the latter medium the fungus grew without any pigmentation at all.

A thorough description of the physiology of *M. persicolor* has been published by Stockdale (1953).

Microscopy of the culture: This showed numerous sub-globose to widely ellipsoid microconidia

borne *en grappe* or more rarely *en thyrese*. Macroconidia, which were less common, were often terminally borne, fusiform, thin-walled, 5–9 celled and had a verrucous surface. In addition, numerous spiral and racquet hyphae were observed.

DISCUSSION

For half a century *M. persicolor* was regarded as an anthropophilic dermatophyte.

Not until English published her studies of the dermatophyte flora on small wild mammals was it possible to classify this fungus among the zoophilic dermatophytes (English, 1966, 1967, 1968; English & Southern, 1967).

The findings made within the past few years of *M. persicolor* in soil are too few to alter the views on the ecological status of this species.

Human lesions look like other cases of tinea circinata caused by zoophilic dermatophytes. The lesions are usually mild, so only a small proportion would reach dermatology clinics and hence be recorded in the literature.

Out of the 142 cases that I have encountered in the literature, the site is stated in 58 (Table 3). Most of the lesions have affected the feet (41%), thereafter the hand, including the forearm (22%). In both sites the nails may be involved. Involvement of the scalp is uncommon, and the same applies to tinea barbae (Duncan, 1945) and tinea cruris (totalling 7%). Kerion formation is extremely rare (Vanbreuseghem, 1949) in contrast to the finding in other zoophilic dermatophyte infections e.g. by *Microsporum canis* and *Trichophyton verrucosum*.

From the therapeutic point of view *M. persicolor* infections seldom give rise to problems, and as is apparent from Table 2 the lesions yield to the current antimycotics.

Regarding nomenclature *M. persicolor* has been a vagrant, having numerous synonyms and constantly changing between being interpreted as a species or a variety. For a long time it was known as *Trichophyton persicolor*. It was not until Stockdale (1967) demonstrated the perfect state *Nannizzia persicolor* Stockdale 1967 that the nomenclature became fixed. Quite recently it has been found that *Nannizzia quinckeana* Balabanov & Schick, 1970, is synonymous with *N. persicolor* (Collinge & Stockdale, 1973; Padhye & Ajello, 1974).

TABLE 3. Site in 58 published cases of *Microsporum persicolor* infections plus the present 12 cases, a total of 70

Site	No. of patients	Author
Scalp	2	Sagher (1947), Spada & Zonchello (1952)
Face	5	Duncan (1945), Milochevitch (1934), Padhye <i>et al.</i> (1973), Sabouraud (1910)
Trunk	5	Blank & Ereaux (1964)
Forearm	3	Milochevitch (1934)
Hand	15	Duncan (1945), Keller (1926), Sabouraud (1910)
Genital region	1	Montpellier & Matamoros (1927)
Lower leg	4	Blank (1955)
Foot	29	Doby <i>et al.</i> (1962), Doby-Dubois <i>et al.</i> (1961), Padhye <i>et al.</i> (1973), Ueckert (1959), Wissel (1956)
Nails	6	Alexander (1922), Bruhns & Alexander (1928), Conti-Diaz (1964), Rieth (1956)

REFERENCES

- ALEXANDER, A. (1922) (a) Statistik der Pilzflora 1919 und 1920. *Trichophyton persicolor*; (b) Interdigitale Soor-mycose; (c) Lichen chronicus Vidal. *Archiv für Dermatologie und Syphilis*, **138**, 410.
- BLANK, F. (1955) Dermatophytes of animal origin transmissible to man. *The American Journal of the Medical Sciences*, **229**, 302.
- BLANK, F. & EREAUX, L.P. (1964) Die Dermatophytenflora der Provinz Quebec. *Der Hautarzt*, **15**, 670.
- BLASCHKE-HELLMESSEN, R. (1975) Statistischer Bericht über die Dermatophytenflora bei Dermatomykosen in der DDR von 1967 bis 1971. *Dermatologische Monatsschrift*, **161**, 433.
- BRUHNS, C. & ALEXANDER, A. (1928) Allgemeine Mykologie In: *Handbuch der Haut- und Geschlechtskrankheiten* (Ed. by J. Jadassohn). Vol. 2.
- COLLINGE, C.A. & STOCKDALE, P.M. (1973) A re-evaluation of *Nannizzia quinckiana* Balabanov & Schick. *Sabouraudia*, **11**, 242.
- CONTI-DIAZ, I.A. (1964) Estudio micológico de 85 casos de onicopatías. *Anales de la Facultad de Medicina de Montevideo*, **49**, 535.
- DOBY, J.-M., ÉON, A. & DOBY-DUBOIS, M. (1962) Griseofulvine et thérapeutique des épidermomycoses inter-digitales du pied par dermatophytes. *Bulletin de la Société de Pathologie Exotique et de Ses Filiales*, **55**, 81.
- DOBY-DUBOIS, M., ÉON, A. & DOBY, J.M. (1961) Les épidermomycoses dermatophytiques interdigitales du pied en Bretagne. Deuxième enquête effectuée dans quelques milieux sportifs. *Bulletin de la Société de Pathologie Exotique et de Ses Filiales*, **54**, 1272.
- DUNCAN, J.T. (1945) A survey of fungous diseases in Great Britain. *British Medical Journal*, **ii**, 715.
- ENGLISH, M.P. (1966) *Trichophyton persicolor* infection in the field vole and pipistrelle bat. *Sabouraudia*, **4**, 219.
- ENGLISH, M.P. (1967) The nature of *Trichophyton persicolor* infection in the bank vole and the interpretation of the results of sampling techniques. *Sabouraudia*, **5**, 295.
- ENGLISH, M.P. (1968) Ringworm in wild mammals. *Journal of Zoology*, **153**, 556.
- ENGLISH, M.P. & SOUTHERN, H.N. (1967) *Trichophyton persicolor* infection in a population of small wild mammals. *Sabouraudia*, **5**, 302.
- GRIGORIU, D. & BROU, A. (1975) La flora fongique isolée à Lausanne de 1967 à 1970. *Mykosen*, **18**, 505.
- HOUIN, R., ROUGUET-CAMPANA, Y., FICHOUX, Y. LA, LANCASTRE, R., BAZIN, J.C., DENIAU, M. & BOLOGNINI, J. (1972) Isolement de *Trichophyton mentagrophytes* (Robin) Blanchard 1896, *Nannizzia persicolor*, Stockdale 1967 et *Trichophyton terrestré*, Durie et Frey 1957, du pelage de rongeurs. Essai D'interprétation écologique. *Annales de Parasitologie Humaine et Comparée*, **47**, 421.
- KARRENBERG, C.L. (1928) Die norddeutsche Pilzflora. Ergebnisse eigener Untersuchungen mit Bemerkungen über die Epidemiologie der Dermatomykosen speziell in Hamburg. *Dermatologische Wochenschrift*, **87**, 1927.
- KELLER, P. (1926) Zur Klinik der Hyphomykosen, insbesondere der dyshidrosiformen Epidermophytien. Zugleich ein Beitrag zur regionären Verbreitung der Hyphomyzeten: die Pilzflora Oberbadens. *Dermatologische Zeitschrift*, **49**, 33.
- MARIAT, F., CHATELAIN, F. & ROUFFAND, M.A. (1975) Flora dermatophytique des petits mammifères sauvages en Alsace, Résultats définitifs portant sur près de 4000 animaux. *Bulletin de la Société Française de Mycologie Médicale*, **4**, 211.
- MILOCHEVITCH, S. (1934) Contribution à l'étude du *Trichophyton persicolor*. *Annales de Parasitologie*, **12**, 289.
- MONTPELLIER, J. & MATAMOROS, P.J. (1927) Trichophytic pubocurale due à '*Trichophyton persicolor*'. *Bulletin de la Société de Dermatologie et de Syphiligraphie*, **34**, 128.
- PADHYE, A. & AJELLO, L. (1974) Further observations on *Nannizzia persicolor* (= *N. quinckiana*). *Sabouraudia*, **12**, 362.
- PADHYE, A.A., BLANK, A., KOBLENZER, P.J., SPATZ, S. & AJELLO, L. (1973) *Microsporum persicolor* infection in the United States. *Archives of Dermatology*, **108**, 561.
- PÄTILÄ, R. (1945) Untersuchungen über die Dermatophyten und die von ihnen hervorgerufenen Krankheiten in Finland, Helsinki.
- RIETH, H. (1956) Nachweis und Einteilung der Dermatophyten unter Auswertung des Krankengutes der Universitäts-Hautklinik, Hamburg von 1951-1955. *Dermatologische Wochenschrift*, **133**, 633.
- SABOURAUD, R. (1910) *Les Teignes*. Masson, Paris.
- SAGHER, F. (1947) The laboratory aspect of fungous diseases of the skin and hair. *Acta Medica Orientalis*, **6**, 68.
- SCHÖNBORN, C., SEIFERT, S., BRAUN, W. & SCHMORANZER, H. (1971) Untersuchungen über Hautpilzen von Zootieren. *Zoologische Garten*, **41**, 7.
- SPADA, C. & ZONCHELLO, S.A. (1952) Studio delle variazioni de terminatesi nella flora dermatomicetica di Cagliari nell' ultimo quinquennio. *Rassegna Medica Sarda*, **3**, 149.

- STOCKDALE, P.M. (1953) Requirements for the Growth and Sporulation of *Trichophyton persicolor*. *Journal of General Microbiology*, **8**, 434.
- STOCKDALE, P.M. (1967) *Nannizzia persicolor* sp. nov., the perfect state of *Trichophyton persicolor* Sabouraud. *Sabouraudia*, **5**, 355.
- SYLVEST, B. (1949) The incidence of dermatophytes in Denmark. *Acta dermato-venereologica*, **29**, 225.
- UECKERT, H. (1959) Beitrag zur Systematik der Dermatophyten. *Zentralblatt für Bakteriologie, Parasitenkunde Infektionskrankheiten und Hygiene*, **176**, 102.
- VANBRESEGHEN, R. (1949) Contribution a la connaissance de *Ctenomyces persicolor* apport d'un cas personnel. *Annales de Parasitologie*, **24**, 124.
- WALKER, J. (1950) The dermatophytoses of Great Britain. Report of a three year's survey. *British Journal of Dermatology*, **62**, 239.
- WISSEL, K. (1956) Studien zur Epidemiologie und Therapie der Fussmykosen. *Dermatologica*, **113**, 156.

Correlations between epithelial cells and bacterial populations in bacteriological skin samples

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SUMMARY

The purposes of this study were to determine whether the variability in populations of skin bacteria observed between individuals is related to the number of epithelial cells removed during the skin scrubbing procedure and also whether any particular group of microorganisms can be directly associated with epithelial cells. Foreheads of 31 subjects were sampled using the cup scrubbing method, and bacterial populations were quantified as: total anaerobes, aerobes, *Propionibacterium* spp., aerobic coryneforms, and *Micrococcaceae*. Epithelial cells were counted in a haemocytometer. Correlation coefficients were positive between total bacterial populations and epithelial cell counts, with the largest values of 0.64 for *Propionibacterium* spp., 0.58 for *Micrococcaceae*, and 0.15 for aerobic coryneforms. High epithelial cell counts were always associated with high bacterial populations, but high bacterial counts occurred, in some instances, with low epithelial cell counts; epithelial cells are not, therefore, responsible for variations in bacterial populations in all cases. Stained smears of epithelial cells from subjects from whom aerobic coryneforms were not cultured showed microcolonies of presumptive anaerobic diphtheroids, closely associated with epithelial cells. These findings and the high correlation between *Propionibacterium* spp. and epithelial cells suggests a re-examination of the location of anaerobes in the stratum corneum.

Individual variation in bacterial populations of human skin has long been recognized. Evans (1975) has recently documented the fact that certain individuals have high bacterial populations while others have low counts, and this is a stable characteristic; but the reasons for this have not been identified. The hypothesis that the physiology of the skin provides a chemical environment which is peculiar to each individual and supports a greater or lesser bacterial population appears to be favoured among theoretical possibilities; owing to the complexity of the system, however, the active components have not been identified.

The hypothesis is presented here that variations observed in bacterial populations from skin are

related to the number of epithelial cells removed during the skin sampling procedure, which would mean that the physical properties of the stratum corneum may contribute to variations in bacterial populations observed between individuals. The depth of the stratum corneum, or variations of the intracellular space, may provide for a greater or lesser physical space for the formation of micro-colonies.

To investigate this hypothesis, the numbers of epithelial cells in skin scrubbing samples have been correlated with bacterial populations; the groups included are: total aerobic and anaerobic populations, total *Propionibacterium* spp., total *Micrococcaceae*, and total aerobic diphtheroids.

MATERIALS AND METHODS

Subjects. Thirty-one volunteers, males and females, all except one between 20 and 30 years of age, were sampled.

Sampling. The forehead was sampled using the cup scrubbing method (Williamson, 1965). The same operator obtained all samples to minimize variation due to scrubbing pressure. Two ml of phosphate buffered saline pH 7.2 containing 0.1% Triton X 100 was divided into two aliquots for scrubbing; these were subsequently pooled, vortexed, diluted by 10-fold steps in phosphate buffered saline, and inoculated on duplicate plates of Casman's sheep blood agar using the drop plate method. One set of plates was incubated aerobically and the other anaerobically using the Gaspak method at 37°C for 3 days for aerobic cultures and 1 week for anaerobic plates.

Microbiology. The following groups of organisms were quantified: total aerobic populations, total anaerobes, total *Propionibacterium* spp., total *Micrococcaceae*, and total aerobic coryneforms. Populations were expressed as $\log_{10}$ colony forming units (CFU)/cm² of skin. Complete speciation of *Micrococcaceae* was not attempted. These organisms were designated as either *Staphylococcus* spp. or *Micrococcus* spp. by the Liefson O/F test (Hugh & Liefson, 1963), recognizing that in some instances this test might not provide a clearcut distinction. Facultative anaerobes were identified by subculture for aerobic growth and excluded from the total anaerobic count. *Propionibacterium* spp. were identified by their strict anaerobic growth, Gram stain morphology, and catalase reactions.

Epithelial cell determinations. Epithelial cells were counted in a Spencer haemocytometer using the Neubauer method.

Statistics. Correlation coefficients were determined between epithelial cells and the population groups described above using the Pearson Correlation Statistic.

Staining of epithelial cells. An additional skin scrubbing sample was taken from four subjects with both a high epithelial cell count and bacterial populations for light microscopy. The sample was centrifuged and washed in phosphate buffered saline and the sediment was heat fixed on microscope slides. Preparations were stained using the Brown & Brenn (1931) method.

RESULTS

The distribution of bacterial populations obtained from skin samples is compared with that of epithelial cells in Fig. 1. The majority of subjects had high total bacterial populations ranging from $\log_{10}$ 5.0 to 8.0 CFU/cm² of skin. There were no subjects with populations lower than $\log_{10}$ 3.3

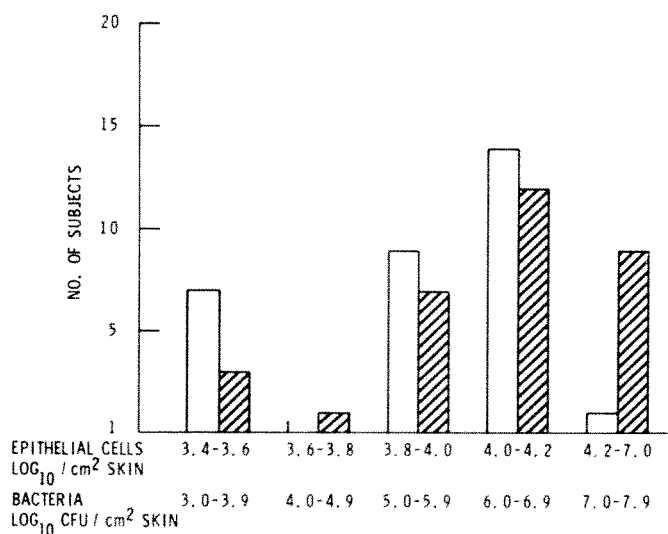


FIGURE 1. Distribution of epithelial cell counts (open columns) and total bacterial populations (hatched columns) in the skin scrubbing samples from the forehead in 31 normal subjects.

TABLE 1. Bacterial populations from skin of the forehead in 31 subjects

Bacteria isolated	Frequency of isolation (%)	Geometric mean ($\log_{10}$ CFU/ cm^2 skin)	s.d.
<i>Propionibacterium</i> spp.	100	6.0	1.24
<i>Micrococcaceae</i> spp.	100	4.1	1.33
Aerobic coryneforms	38	1.3	1.78

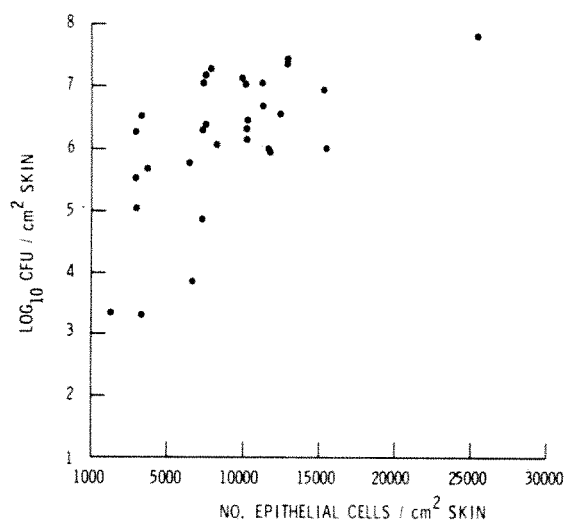


FIGURE 2. Relationship between epithelial cell counts and total populations of aerobic and anaerobic bacteria in skin scrubbing samples in 31 subjects. Total bacteria correlation coefficient 0.6486.

TABLE 2. Correlation coefficients between epithelial cell populations and bacteria in skin scrubbing samples

Bacterial groups	Correlation coefficient	Significance <i>P</i> value
Total aerobes	0.60	0.001
Total anaerobes:		
(<i>Propionibacterium</i> spp.)	0.64	0.001
<i>Micrococcaceae</i>	0.58	0.001
<i>Staphylococcus</i> spp.	0.11	0.273
<i>Micrococcus</i> spp.	0.39	0.015
Aerobic coryneforms	0.15	0.219

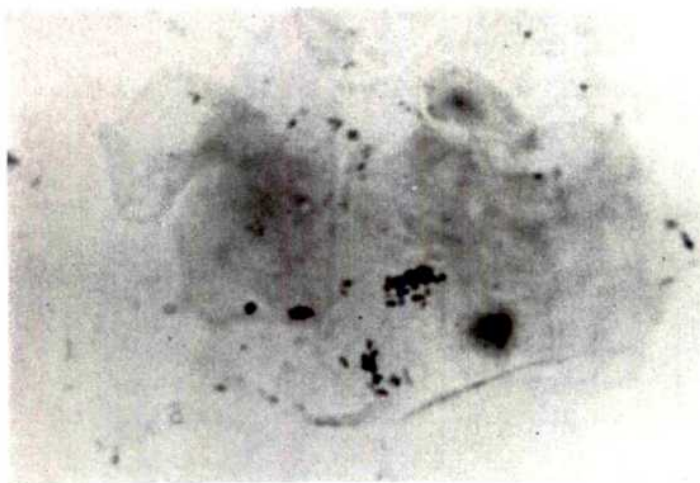


FIGURE 3. Epithelial cells from a skin scrubbing sample showing microcolonies of bacteria with coryneform morphology closely associated to the epithelial cells.

CFU/cm² of skin and only four subjects with populations lower than log₁₀ 5.0 CFU/cm² of skin. Fig. 1 also shows that varying numbers of epithelial cells are removed from subjects during the skin scrubbing procedure ranging from 2631/cm² of skin to 25,668/cm² of skin.

The bacterial populations were comprised of the following genera: *Propionibacterium* spp., *Micrococcaceae*, aerobic coryneforms, and *Peptococcus* spp. The latter were present in so few subjects that they were not included in the statistical correlations. The relative populations of the different genera are shown in Table 1. *Propionibacterium* spp. were isolated with the greatest frequency and had the highest populations. Aerobic coryneforms had the lowest populations and frequency of isolation (38.7%), while *Micrococcaceae* spp. held an intermediate position. One hundred percent of the subjects had *Micrococcaceae* in their skin samples; the frequency of isolation was 97.0% for those designated as *Staphylococcus* spp. and 80.6% for *Micrococcus* spp.

The highest correlation coefficient, 0.65 ($P = 0.001$), occurred between total bacterial and epithelial cell populations. The distribution of counts (Fig. 2) shows that, although the subjects with the highest

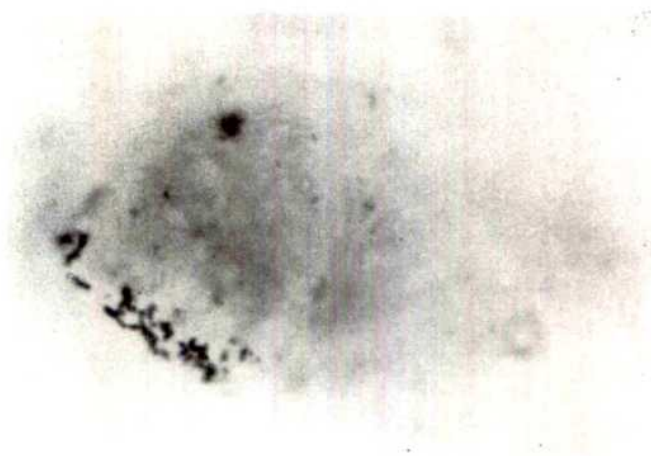


FIGURE 4. Epithelial cells from skin scrubbing samples showing bacteria with coryneform morphology aligned along the edge of the epithelial cell.

and lowest epithelial cell counts had the highest and lowest bacterial populations, respectively, there were five subjects who had low epithelial cell counts and high bacterial populations, and five more who had relatively low epithelial cell counts and high microbial populations. None of the samples with numerous epithelial cells, however, had low bacterial populations.

The correlation coefficients and their significance for the different groups of bacteria studied are shown in Table 2.

Visual examination of the sedimented, washed and stained skin scrubbing sample is shown in Figs 3 and 4. Numerous Gram positive coryneform bacteria can be seen closely associated with the epithelial cells, both apparently on the surface (Fig. 3), as well as aligned along the edges (Fig. 4). There were no aerobic coryneforms cultured from the subject from whom this sample was obtained.

DISCUSSION

The correlation coefficient of 0.65 obtained from epithelial cell counts and total bacterial populations is sufficiently high to conclude that the numbers of bacteria removed from skin is related to the numbers of epithelial cells removed. It does not appear to be sufficiently high, however, to account completely for variation in microbial populations of skin observed between individuals. There were five subjects in the group of 31 with bacterial populations ranging from $\log_{10}$ 5.0 to 6.51 CFU/cm² of skin with epithelial cell counts less than 5000/cm² of skin. On the other hand, none of the subjects with numerous epithelial cells had low bacterial populations.

The variation observed in the numbers of epithelial cells removed from different individuals using an identical scrubbing procedure suggests that the stratum corneum varies in depth or number of cells. These different physical properties might contribute to varying growth conditions, by providing space for bacterial growth, as well as the number of dead cells from which nutrients may be derived. The concept of different physical properties of the stratum corneum affecting the growth of bacteria present is not as naive as it might seem; large bacterial populations are associated with a number of exfoliative conditions, among the most common being dandruff (Priestley & Savin, 1976); and higher

than normal bacterial populations have been described in psoriatic plaques (Aly, Maibach & Mandel, 1976), although the latter condition cannot be considered normal and other factors may be present.

A finding of particular interest in this study is the high correlation coefficient between the anaerobic *Propionibacterium* spp. and epithelial cells. This observation indicates that a re-evaluation of the location of anaerobic bacteria in the skin is in order. Although scanning electron microscopy has shown microcolonies on the skin surface (Noble & Somerville, 1974) which have also been recorded using light and transmission electron microscopy (Montes & Wilborn, 1969, 1970), histological studies tend to support the view that the highest populations of skin bacteria are located not in the stratum corneum, but in the infundibulum of the hair follicle (Kligman, 1965; Montes & Wilborn, 1969, 1970; Baxby & Woodroffe, 1965). Microbiological studies by Röckl & Müller (1959) and Updegraff (1964), using a tape skin stripping culture method, concluded that 75% of aerobic skin bacteria are located on the top layer of the stratum corneum. Studies on dispersal of microorganisms from desquamated skin (Noble, 1975) have also utilized only aerobic culture methods, and hence it is still generally believed that the aerobic flora is principally on the surface, while the anaerobic flora is mainly in the infundibulum of the hair follicle. Sommerville & Noble (1973) compared contact plates with scrub samples to determine microcolony size of both aerobic and anaerobic populations; and, although they obtained over 100 anaerobic colonies in 3 out of 5 subjects, using contact plates, they concluded that the anaerobes were related to an opening of a hair follicle.

The results present here suggested that large populations of anaerobes are closely associated with epithelial cells, which raises the question of the mechanism of lower oxygen tension to permit growth. Possibly, the non-viable epithelial cells occurring in the stratum corneum provide a lower oxygen tension than the viable tissue cells of the infundibulum; thus, the thicker the stratum corneum, the greater the 'dead' space for growth.

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REFERENCES

- ALY, R., MAIBACH, H.I. & MANDEL, A.M. (1976) Bacterial flora in psoriasis. *British Journal of Dermatology*, **95**, 603.
- BAXBY, D. & WOODROFFE, R.C.S. (1965) The location of bacteria in skin. *Journal of Applied Bacteriology*, **28**, 316.
- BROWN, J.R. & BRENN, L. (1931) A method for the differential staining of gram positive and gram negative bacteria. *Bulletin of Johns Hopkins University*, **48**, 69.
- EVANS, C.A. (1975) Persistent individual difference in the bacterial flora of the skin of the forehead: numbers of *Propionibacteria*. *Journal of Investigative Dermatology*, **64**, 42.
- HUGH, R. & LIEFSON, E. (1953) The taxonomic significance of fermentive versus oxidative metabolism of carbohydrates by various Gram negative bacteria. *Journal of Bacteriology*, **66**, 24.
- KLIGMAN, A.M. (1965) The bacteriology of normal skin. In: *Skin Bacteria and their Role in Infection* (Ed. by H.I. Maibach and G. Hildick-Smith), p. 12. McGraw-Hill, New York.
- MONTES, L.F. & WILBORN, W.H. (1969) Location of bacterial skin flora. *British Journal of Dermatology*, **81**, Suppl. 1, 23.
- MONTES, L.F. & WILBORN, W.H. (1970) Anatomical location of normal skin flora. *Archives of Dermatology*, **101**, 145.
- NOBLE, W.C. (1975) Dispersal of skin microorganisms. *British Journal of Dermatology*, **93**, 477.
- NOBLE, W.C. & SOMERVILLE, D.A. (1974) Cutaneous populations. In: *Microbiology of Human Skin*, p. 61. W.B. Saunders, London.
- PACHTMAN, E.A., VICHER, E.E. & BRUNNER, M.J. (1954) The bacteriologic flora in seborrheic dermatitis. *Journal of Investigative Dermatology*, **22**, 389.

- PACHTMAN, E.A., VICHER, E.E. & BRUNNER, M.J. (1954) The bacteriologic flora in seborrheic dermatitis. *Journal of Investigative Dermatology*, **22**, 389.
- PRIESTLY, G.C. & SAVIN, J.A. (1976) The microbiology of dandruff. *British Journal of Dermatology*, **94**, 469.
- RÖCKL, H. & MÜLLER, E. (1959) Beitrag zur Lokalisation der Mikroben der Haut. *Archiv für klinische und experimentelle Dermatologie*, **209**, 13.
- SOMERVILLE, D.A. & NOBLE, W.C. (1973) Microcolony size of microbes on human skin. *Journal of Medical Microbiology*, **6**, 323.
- UPDEGRAFF, D.M. (1964) A cultural method of quantitatively studying microorganisms of skin. *Journal of Investigative Dermatology*, **43**, 129.
- WILLIAMSON, P. & KLIGMAN, A.M. (1965) A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology*, **45**, 498.

An assessment of (1) cyproterone acetate and (2) ethinyl oestradiol and lynoestrenol (Minilyn) in the treatment of idiopathic hirsutism

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SUMMARY

The administration of cyproterone acetate can be effective in the treatment of 'idiopathic' hirsutism. In 5 similarly affected patients treated with ethinyloestradiol and lynoestrenol (Minilyn) the rate of hair growth was reduced in only two, even though the serum total testosterone was reduced in each instance.

Many hirsute patients after extensive investigation have been shown to have no gross endocrine abnormality. It is this group which for convenience has been labelled as having 'idiopathic' hirsutism.

Cyproterone acetate is a very powerful progestogen and antiandrogen. Hammerstein & Cupceancu in 1969 demonstrated the possible effectiveness of this drug in the treatment of hirsutism using a 'reversed two phase therapy' with ethinyloestradiol. Further reports such as those of Ismail *et al.* (1974) and Ebling *et al.* (1977) have confirmed these findings. This form of therapy would seem to provide an aesthetic approach to the treatment of the severely affected woman.

Several reports such as those of Casey *et al.* (1966), Ettinger *et al.* (1973), and Hancock & Levell (1974), have shown that oral contraceptives may also be of value in the treatment of hirsutism. It has been suggested that these preparations work by suppressing ovarian steroidogenesis, influencing adrenal output at the pituitary level, or by a direct antiandrogenic effect at the hair follicle. The present study was therefore planned to assess objectively hair growth in hirsute women receiving these drugs over a 6-month period.

MATERIALS AND METHOD

The 20 women included in the trial were between 13 and 46 years old when first seen. All had first noticed hirsutism in their teens or twenties. The patients' 'hormonal hair count' was quantitatively assessed using the method of Ferriman & Gallwey (1961) and no one was included in the series with a count of less than six.

TABLE 1. Therapy

Number of patients	Drugs	Days in menstrual cycle
5	Ethinylloestradiol 50 µg Lyncestrenol 2.5 mg 'Minilyn'	5-26
8	Cyproterone acetate 100 mg	5-14
	+	
	Ethinylloestradiol 50 µg	5-21
7	Cyproterone acetate 100 mg	5-14

Those patients who had at some time previously been taking oral contraceptives were given 'Minilyn' for the trial period. The other 15 women were given cyproterone acetate. If there was any concern about possible pregnancy ethinylloestradiol was included in their regime (Table 1).

Hair growth was measured on three separate occasions before commencing therapy, and then at frequent intervals during therapy. The site chosen was the most severely affected area—usually the face, abdomen or thigh. The patient was instructed to shave this area 7 days before being seen at the clinic. The 7-day growth of hair was then shaved using a disposable Gillette razor and collected on a filter paper previously inserted into a glass funnel. The ten longest hairs were then measured, after being allowed to dry to the fairly constant hospital humidity, using a pocket microscope fitted with graticule scales. The length of each hair was measured to an accuracy of 0.1 mm (Fig. 1) and the broadest diameter to 0.01 mm (Fig. 2). The product of these two measurements gave a comparative mass result.

On each visit the patient brought a 24-h collection of urine, and 10 ml of venous blood was withdrawn. Serum testosterone and androstenedione were measured by radioimmunoassay and individual urinary steroids were estimated by gas-liquid chromatography; the M.R.C. standard method was

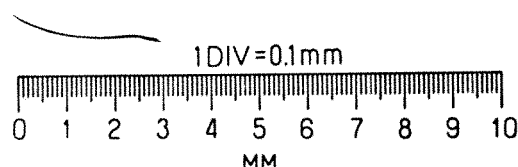


FIG. 1.

FIGURE 1. Scale for measuring length of hair. Example of hair of length 3.20 mm.

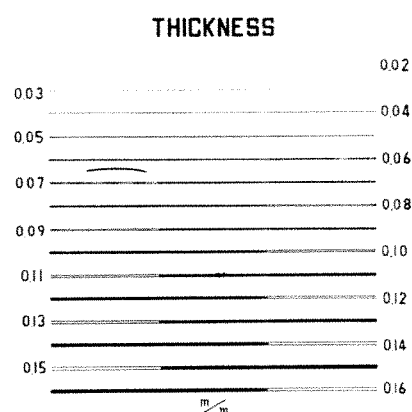


FIG. 2.

FIGURE 2. Scale for measuring diameter of hair, in this case 0.07 mm.

used for 17-oxosteroids, and the method of Few (1961) for 17-hydroxy: corticosteroids. Pregnanetriol was estimated by the method of Fotherby (1960).

Before starting and at the end of the 6-month period of treatment the following laboratory investigations were also carried out: (i) full blood count; (ii) serum urea and electrolytes, aspartate transaminase, total protein and albumin.

RESULTS

The efficacy of treatment was assessed by expressing hair growth during treatment as a percentage of pre-treatment growth.

TABLE 2. Patient No. 11—hair collected from lower abdomen. Each specimen result being the average of the measurements of the 10 longest hairs collected in the area under observation.

	Pre-treatment			During treatment Specimen 7 (mm) Week 14
	Specimen 1 (mm)	Specimen 2 (mm)	Specimen 3 (mm)	
Length of hair in 7 days	3.76	3.81	3.41	3.09
Length per day	0.54	0.54	0.49	0.44
Broadest diameter	0.109	0.099	0.144	0.051
Comparative daily mass result	0.059	0.054	0.056	0.0224

Average pre-treatment (comparative daily mass) = 0.0563, i.e. 100%

During treatment = 0.0224, i.e. 39.8%

The effect on hormonal hair growth in the patients given lynoestrenol and ethinyloestradiol is expressed in Table 3. Of the 5 patients taking this combination 2 defaulted before the end of the trial period (A and E), one of whom (A) had shown a measurable reduction in hair growth. Of the remaining 3 patients only one (C) had demonstrated a reduction in hair growth. (B) and (D) had not altered their hair growth rates at all.

The only significant change ($P < 0.001$) in biochemical parameters measured during treatment with lynoestrenol and ethinyloestradiol was the reduction in plasma total testosterone (Fig. 3).

Unlike 'Minilyn' the effect on hair growth of cyproterone acetate, with or without ethinyloestradiol, was pronounced. As there was no significant difference in results between the two groups of patients taking cyproterone acetate alone or with ethinyloestradiol, the results of these two groups have been put together (Table 4).

Fourteen of the patients responded to treatment with a reduction in hair growth of at least 30%, three of these showing a return to vellus hair in the areas under observation. One patient (No. 8) failed to show any change in hair growth rates during the trial period.

An interesting feature was the increase in hair growth noticed in those patients seen in the initial phase of therapy. This was followed during the next 8-week period by the maximum rate of reduction in hair growth before a relatively stable rate of hair growth was reached.

There was no significant change in serum total testosterone, androstenedione, or any of the urinary steroids during the final period. Also, no abnormal findings were demonstrated in the pre and post-treatment routine haematological and biochemical investigations.

TABLE 3. Effect of treatment with Ethinyloestradiol and Lynoestrenol in 5 patients. Hair growth expressed as comparative daily mass and percentage of pre-treatment value

Site of sample	Pre-treatment comparative mass result	Weeks of therapy													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
A. Upper lip	0.0101 100%						0.00836 83%					0.00624 62%			
B. Lower abdomen	0.0346 100%				0.0412 119%					0.038 110%					
C. Chin	0.0198 100%	0.0186 94%			0.015 76%							0.0214 108%			
D. Chin	0.0404 100%		0.0346 86%					0.0434 107%			0.0288 104%			0.0433 107%	
E. Lower abdomen	0.0278 100%						0.0252 91%								

Site of sample	Pre-treatment comparative mass result	Weeks of therapy													
		15	16	17	18	19	20	21	22	23	24	25	26	27	28
A. Upper lip	0.0101 100%														
B. Lower abdomen	0.0346 100%		N					N			0.033 95%				
C. Chin	0.0198 100%			0.0189 95%						N				0.0104 53%	
D. Chin	0.0404 100%				N					0.0404 100%					
E. Lower abdomen	0.0278 100%														

N = No specimen obtained.

TABLE 4. Effect of treatment with Cyproterone Acetate $\pm$ Ethinyloestradiol on hair growth in 15 patients. Results shown as comparative daily mass of hair and percentage of pre-treatment values

Site of sample	Pre-treatment comparative mass result	Weeks of therapy												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1. Upper lip	0.0324 100%			0.034 105%			0.0198 61%			0.0202 63%				
2. Thigh	0.0211 100%	N					0.0241 114%					0.0149 71%		
3. Lower abdomen	0.0473 100%		0.047 99%						0.032 68%					0.0246 52%
4. Upper lip	0.008 100%	0.00722 90%								N				0.00406 51%
5. Lower abdomen	0.054 100%	0.054 100%					0.0045 56%	0.0281 52%			0.0326 60%			0.0336 62%
6. Lower abdomen	0.0111 100%				0.009 81%			0.0069 62%				V		
7. Chin	0.055 100%				0.0526 96%		0.0533 97%				0.0494 90%			
8. Chin	0.0598 100%						0.0505 84%			0.0685 115%				0.0605 101%
9. Chin	0.063 100%			N				0.0568 90%					0.0477 76%	
10. Chin	0.0526 100%	0.068 128%					0.0505 95%		0.0318 61%					
11. Lower abdomen	0.0563 100%				0.047 83%			0.0272 48%				0.0149 26%		
12. Thigh	0.0380 100%		0.0410 108%					0.0372 98%		0.0248 65%				N
13. Chin	0.025 100%			0.026 104%				0.0298 119%				0.0195 78%		
14. Chin	0.038 100%			0.041 108%					0.0268 71%					0.0392 77%
15. Lower abdomen	0.0221 100%	0.0265 120%		0.0382 173%		N						0.013 59%		

TABLE 4. (cont.)

Site of sample	Pre-treatment comparative mass result	Weeks of therapy													
		15	16	17	18	19	20	21	22	23	24	25	26	27	28
1. Upper lip	0.0324 100%	0.0190 58%				0.0170 53%						0.0252 81%			0.0174 54%
2. Thigh	0.0211 100%			N				0.0121 57%					N		
3. Lower abdomen	0.0473 100%									0.022 47%	V				
4. Upper lip	0.008 100%			0.00378 47%			0.0030 38%								
5. Lower abdomen	0.054 100%			0.0288 53%		0.0348 64%				0.0308 57%				0.0265 49%	
6. Lower abdomen	0.0111 100%									V					
7. Chin	0.055 100%	0.0549 100%				0.043 78%					0.0351 64%				
8. Chin	0.0598 100%			0.0620 103%					0.0656 110%					0.0625 105%	
9. Chin	0.063 100%		0.0378 60%				0.0496 79%				0.0343 55%				0.0838 61%
10. Chin	0.0526 100%				0.0414 78%					0.025 48%					
11. Lower abdomen	0.0563 100%	0.0224 40%					0.020 36%				0.0198 35%				0.0200 36%
12. Thigh	0.0380 100%					0.0225 59%					N				
13. Chin	0.025 100%	0.0171 69%			V						V				
14. Chin	0.038 100%							0.0187 49%							
15. Lower abdomen	0.0221 100%	0.0125 57%				0.012 54%					0.0085 38%				

N = no specimen obtained.
V = vellus hair (judged by clinical observation).

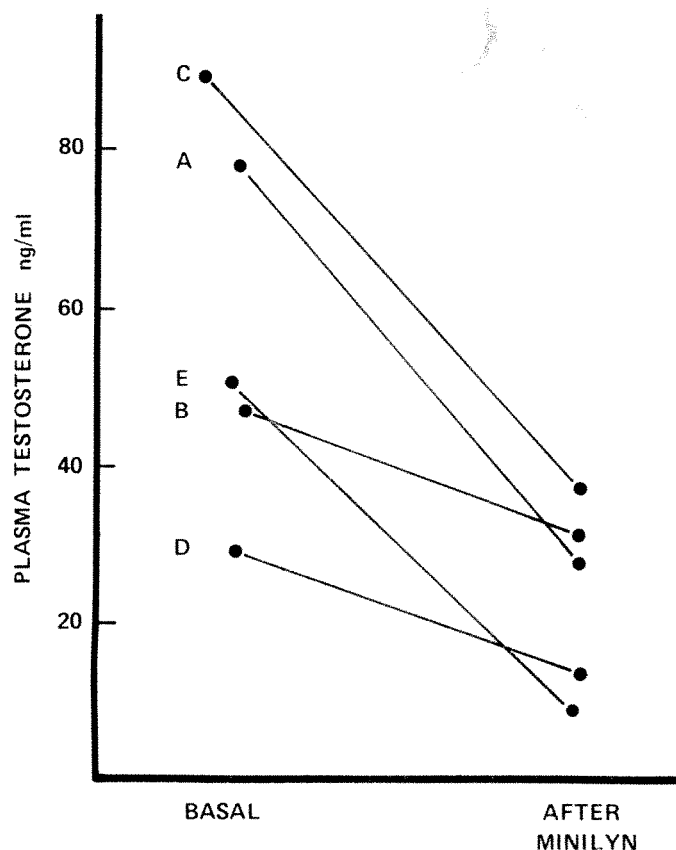


FIGURE 3. Effect on total plasma testosterone in 5 patients treated with ethinyloestradiol and lynoestrenol.

DISCUSSION

One of the main difficulties in a trial such as this is the attainment of objectivity while inconveniencing the patient as little as possible. Hair weighing was considered but was found to be impractical as a greater mass of hair was required to be collected to show significant changes in weighing during treatment. This would of course involve the patient in having an unsightly area of terminal hair for a longer period than for the method used. To measure actual hair volume produced by the follicles was exceptionally time-consuming, so a compromise was used which enabled comparison readings to be made for individual patients before and during treatment.

The results obtained using the oral contraceptive combination of lynoestrenol and ethinyloestradiol were variable and are similar to those in other published reports (Casey *et al.*, 1966; Ettinger *et al.*, 1973; Hancock & Levell, 1974). As the serum testosterone concentration is consistently reduced using 'Minilyn', unlike some other oral contraceptive preparations (Browning & Anderson 1977), any action that 'Minilyn' has in reducing hirsutism would not appear to be related to this effect.

The results obtained using cyproterone acetate show that it is possible to reduce the growth rate of male distribution secondary sexual hair in females.

The characteristics of the hair growth rates during administration of this drug would suggest that

its efficacy is due to a direct blocking action at the hair follicle and would support the conclusions drawn by Neuman & Berswordt-Wallrabe (1966) concerning its action at androgen receptor sites. The effect of cyproterone acetate would therefore be dose-dependent and by varying the dose of this drug according to the clinical effect it should be possible to obtain a more uniform effect in all patients with idiopathic hirsutism.

There was no doubt that subjectively all the women receiving this drug were delighted with the results obtained—even surprisingly the patient in whom no objective evidence of reduction in hair growth was obtained. This is perhaps confirmed by the fact that there were no defaulters in this group of patients as compared with those receiving 'Minilyn'. This also serves as an illustration of the patient-acceptability of the treatment with regard to 'side effects'.

The questions arise of course as to what is the optimum period of time to give a suppressive dose of cyproterone acetate, and what to do after this period? The answer to the former question will depend on the patients' responsiveness to therapy, and to the latter it will probably be necessary to elucidate a low maintenance dose of antiandrogen.

ACKNOWLEDGMENTS

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REFERENCES

- BROWNING, M.C.K. & ANDERSON, J.A.R. (1977) Effect of oral contraceptives on plasma testosterone concentration. *British Medical Journal*, **1**, 107.
- CASEY, J.H., BURGER, H.G., KENT, J.R., KELLIE, A.E., MOXHAN, A., NABARRO, J. & NABARRO, J.D.N. (1966) Treatment of hirsutism by adrenal and ovarian suppression. *Journal of Clinical Endocrinology*, **26**, 1370.
- EBLING, F.J., THOMAS, A.K., COOKE, I.D., RANDALL, V.A., SKINNER, J. & CAWOOD, M. (1977) Effect of cyproterone acetate on hair growth, sebaceous secretion and endocrine parameter in a hirsute subject. *British Journal of Dermatology*, **97**, 371.
- ETTINGER, B., GOLDFIELD, E.G., BURRILL, K.C., VON WERDER, K. & FORSHAM, P.H. (1973) Plasma testosterone stimulation—suppression dynamics in hirsute women. *American Journal of Medicine*, **54**, 195.
- FERRIMAN, D. & GALLWEY, J.D. (1961) Clinical assessment of body hair growth in women. *Journal of Clinical Endocrinology and Metabolism*, **21**, 1440.
- FEW, J.D. (1961) A method for the analysis of urinary 17-hydroxycorticosteroids. *Journal of Endocrinology*, **22**, 31.
- FOTHERBY, K. & LOVE, D.N. (1960) A modified method for the estimation of pregnanetriol in urine. *Journal of Endocrinology*, **20**, 157.
- HAMMERSTEIN, J. & CUPCEANCU, R. (1969) The treatment of hirsutism with cyproterone acetate. *German Medical Monthly*, **14**, 599.
- HANCOCK, K.W. & LEVELL, M.J. (1974) The use of oestrogen/progestogen preparations in the treatment of hirsutism in the female. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **81**, 804.
- ISMAIL, A.A.A., DAVIDSON, D.W., SOUKA, A.R., BARNES, E.W., IRVINE, W.J., KILIMNIK, H. & VAN DER BEEKEN, Y. (1974) The evaluation of the role of androgens in hirsutism and the use of a new anti-androgen 'cyproterone acetate' for therapy. *Journal of Clinical and Endocrinological Metabolism*, **39**, 81.
- M.R.C. COMMITTEE ON CLINICAL ENDOCRINOLOGY MEMORANDUM (1963) A standard method of estimating 17-oxosteroids and total 17-oxogenic steroids. *Lancet*, **i**, 1415.
- NEUMANN, F. & VON BERSWORDT-WALLRABE, R. (1966) Effects of the androgen antagonist cyproterone acetate on the testicular structure, spermatogenesis and accessory sexual glands of testosterone treated hypophysectomised rats. *Journal of Endocrinology*, **35**, 363.

Case Reports

Generalized Hailey-Hailey disease

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SUMMARY

A woman of 60 developed a generalized erythroderma. The case history revealed classical symptoms of Hailey-Hailey disease for 17 years. Light- and electron microscopic investigations confirmed the diagnosis of generalized Hailey-Hailey disease—an extremely rare condition. Two additional cases with widespread clinical manifestations are presented. These observations underline the fact that the whole epidermis is genetically disturbed and may react to various insults with suprabasal acantholysis.

Chronic benign familial pemphigus (Hailey-Hailey) is an autosomal dominant hereditary disease with irregular penetrance (Gschnait, 1973). Sporadic cases have also been reported (Palmer & Perry, 1962). The epidermis reacts to actinic, thermal, chemical and mechanical stimuli with suprabasal acantholysis.

We present three patients to demonstrate that the clinical manifestations of the disease may not be confined to the intertriginous areas, but can affect the entire skin.

Case 1

A 60-year-old female patient, with no relevant family history. Previously she had had measles and scarlet fever as a child and in 1933 had an appendicectomy, and in 1973 an operation for senile cataract. The onset of the eruption dates back to 1960. The disease showed exacerbations in the warm season in the intertriginous areas, the axillae being worst affected. Pruritus was the most distressing subjective symptom. Following unsuccessful local therapy in 1960, surface X-ray therapy was given, which led to a remission. Early in January 1977, the patient noticed pin-head sized spots, at first on the breast, which then gradually spread over the entire body. She was admitted for treatment on 24 February 1977.

Dermatological findings. There was diffuse reddening, lamellar scaling and foci of superficial erosions over the entire integument, with accentuation over the trunk (Fig. 1). There were in addition oozing and crusted erythematous areas in the flexures. Subjectively there was intense itching. Later in the clinical course, grouped vesicles and pustules appeared.

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FIGURE 1. Case 1: generalized exfoliative dermatosis with crusted and oozing intertriginous areas.

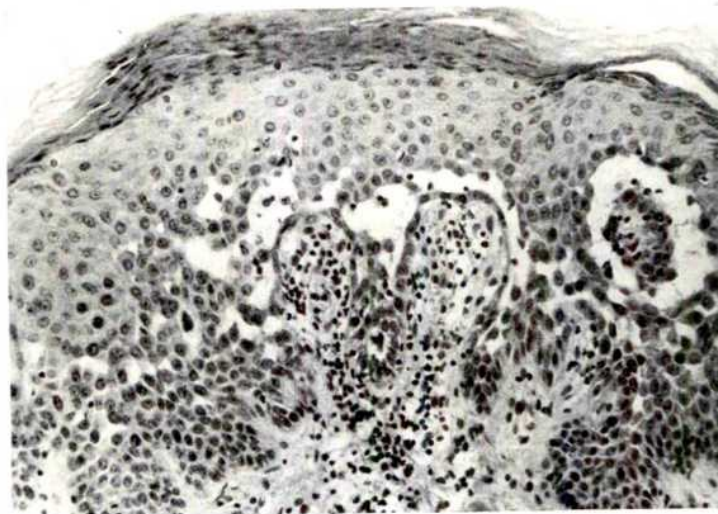


FIGURE 2. Case 1: Biopsy from the abdominal wall showing suprabasal acantholysis, parakeratosis, slight lymphocytic perivascular infiltration of the papillae. Biopsy from the abdominal wall Case 1 (H&E, $\times 1600$).

Laboratory findings. SGPT 24 U/l. Blood count: leukocytosis of $13,200/\text{mm}^3$, ESR 24mm/h. No other abnormalities.

Direct and indirect immunofluorescence. There were no positive findings for IgG.

Microscopy. Two specimens of abdominal skin show clear focal suprabasal clefts with isolated or grouped acantholytic keratinocytes with strongly eosinophilic cytoplasm (Fig. 2). Corps ronds and grains are not observed. There is lamellar hyperkeratosis and patchy parakeratosis. Slight lymphocytic perivascular infiltration in the papillary layer is also seen.

Electron microscopy (abdominal skin). In the region of the acantholytic clefts, the intercellular spaces between the keratinocytes of the spinous and basal layers of epidermis are enlarged. Only the keratinocytes of the basal layer show regular structures towards the basement membrane (Fig. 3). Where there are enlarged intercellular spaces, the surface of the keratinocytes is villous. Though the desmosomes are rarified, they are nevertheless normally constructed (Fig. 4). With increasing enlargement of the intercellular gaps, the desmosomes disappear completely. Detached acantholytic keratinocytes within the vesicles and clefts show a bizarre surface structure with prominent microvillous change (Fig. 5). Partial premature keratinization (dyskeratosis) is absent. It is striking that, in the region of early acantholysis, keratinocytes exhibit tonofilaments oriented circularly around the nucleus in wide formation outside a perinuclear free zone; sometimes these appear in plexiform or whorl-like configuration.



FIGURE 3. Suprabasal vesicle (V), acantholytic keratinocytes (AK) with microvilli-like transformation of the surface. Basal lamina (B) ($\times 5400$).

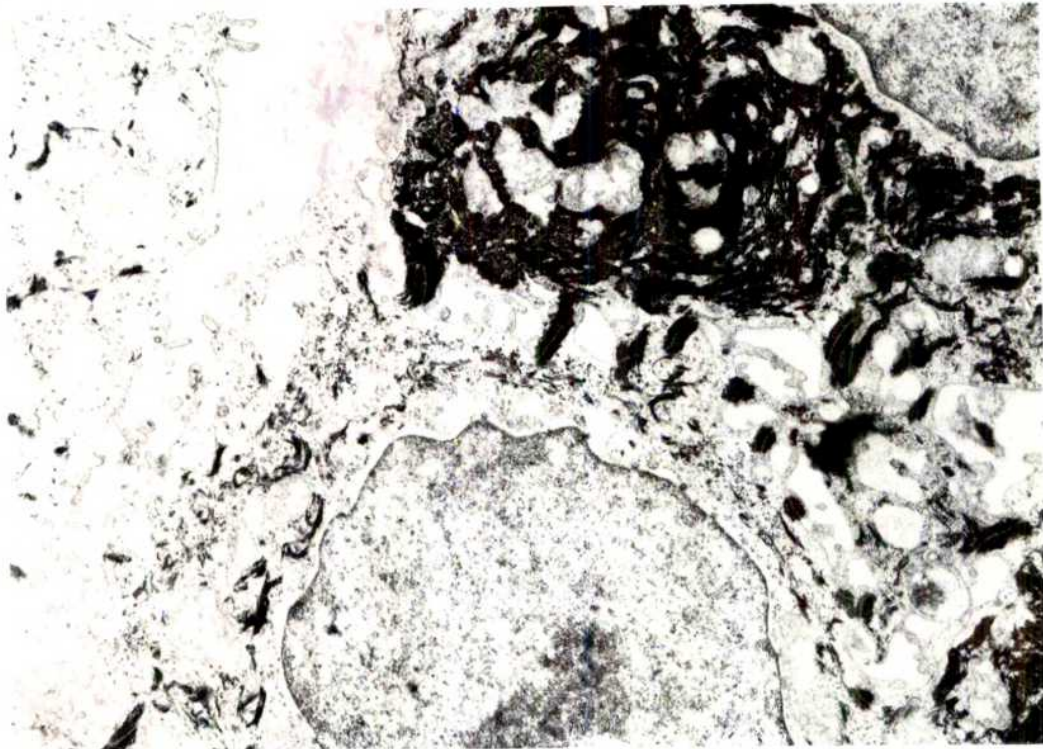


FIGURE 4. Widening of the intercellular spaces, rarefied but regular desmosomes. Whorl-like configuration of bundles of tonofilaments in one keratinocyte (top) ($\times 12,420$).

Therapy and course. She was treated with prednisolone (up to 150 mg/day), and the erythrodermic skin condition rapidly improved. After reduction of prednisolone, there was rapid recurrence with an erythrodermic episode and the appearance of grouped vesicles and pustules. Improvement occurred once again after starting 50 mg of prednisolone/day and oral tetracycline. Later she was given topical gentamicin and steroid therapy and discharged relatively well without systemic corticosteroids. Later on the erythrodermic skin condition with typical oozing, crusted, intertriginous areas recurred and systemic corticosteroids were started once more (20 mg fluocortolone/day). Topical applications of a zinc oxide ointment were also given. Under this regime the patient was, for the most part, clinically symptom free for 3 summer months. The systemic steroid therapy was discontinued, and 6 months have passed without relapse.

Case 2

A 57-year-old male patient with no relevant family history. He has had psoriasis from the age of 19 years. Eight years ago at the age of 49 he developed a distressing widespread oozing and crusted eruption. He was treated for Hailey-Hailey disease at the Department of Dermatology of the University of Hamburg until 1955 (the same patient as presented by Herzberg, 1955). He later moved to Berlin and was admitted for treatment in 1961.

Dermatological findings. There were typical disseminated oozing and crusted erythematous confluent plaques except on the head, hands and feet (Fig. 6).

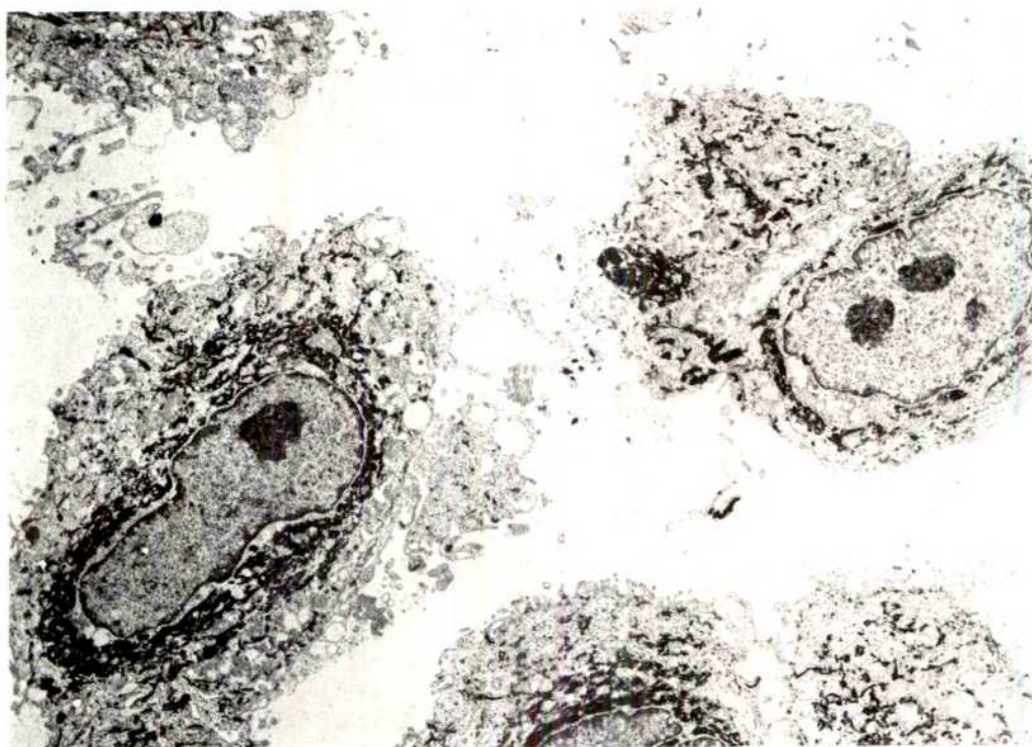


FIGURE 5. Detached acantholytic keratinocytes in the lumen of a suprabasal vesicle. Microvilli-like transformation of cell-surface ($\times 4860$).



FIGURE 6. Case 2: Disseminated typical lesions of Hailey-Hailey disease.

FIGURE 7. Case 3: Lesions of Hailey-Hailey disease in the mammary region.

Histology. Multiple biopsies confirmed the diagnosis by the presence of prominent suprabasal acantholysis.

Therapy and course. The clinical manifestations could only be suppressed by high systemic doses of corticosteroids.

Case 3

A 16-year-old girl without positive family history. Three years ago she developed circumscribed erythematous oozing and partly crusted lesions on the chest, the back and the neck. There was severe itching and occasional vesicles were seen in affected areas. The disease vanished after several weeks. In the past 6 months lesions have once again appeared at the same sites.

Dermatological findings. There were disseminated typical oozing and partly crusted erythematous lesions on the neck, the back and the chest (Fig. 7). Later some vesicles appeared on erythematous areas on the right lower leg.

Histology. Multiple biopsies have shown typical suprabasal acantholytic cleft and vesicle formation.

DISCUSSION

Three patients with disseminated Hailey-Hailey disease are reported in this paper.

The 16 year history of the 60-year-old female patient (Case 1) leaves no doubt about the diagnosis of Hailey-Hailey disease. The occurrence of generalized erythroderma in 1977 is noteworthy. Cases of Hailey-Hailey disease with generalization have very seldom been reported (Herzberg, 1955; Lewis, 1948). In the differential diagnosis, a bullous or vesiculo-pustular variant of Darier's disease should be considered. The intermittently occurring, grouped vesicles and pustules could also support this consideration. The following, however, are against Darier's disease:

(1) The late onset of the disease after 43 years as well as the non-progressive clinical course with predominantly symptom-free intervals.

(2) At no time did keratotic lesions appear, and, in particular, the sites of predilection for Darier's disease were not affected. Pustular eruptions have also been described in cases of Hailey-Hailey disease (Herzberg, 1955; Lyles, Knox & Richardson, 1958).

(3) Histologically, corps ronds and grains could not be observed to the requisite extent (Gschnait, 1973).

The ultrastructural findings show the acantholytic cleft and vesicle formation as they have been described in Hailey-Hailey disease (Gottlieb & Lutzner, 1970; Nürnberger & Müller, 1967; Piérard & Kint, 1969; Wilgram, Caulfield & Lever, 1962). Darier's disease can be differentiated with certainty from Hailey-Hailey disease by electron microscopy (Piérard & Kint, 1969). It cannot be deduced conclusively from the ultrastructural findings that a primary disturbance in the desmosome-tono-fibril complex is the underlying cause of the disease (Wilgram, Caulfield & Lever, 1962). An alteration in the intercellular substance can be postulated as the cause of the villiform configuration and thus of the enlargement of the keratinocyte cytoplasm membrane (Nürnberger & Müller, 1967). The very similar ultrastructural findings in cases of transitory acantholytic dermatosis were likewise interpreted by the first author to describe them (Grover, 1971). Even though this clinical picture (acute occurrence of pruriginous papulovesicles with spontaneous healing in a few weeks) is clearly differentiated from Hailey-Hailey disease (Chalet, Grover & Ackerman, 1977; Pehamberger, Gschnait, Konrad & Holubar, 1977; Wolff, Chalet & Ackerman, 1977), a similar mechanism must be thought of on the basis of the morphological findings.

Pemphigus erythematosus and pemphigus foliaceus could be ruled out immunologically and histologically.

The two additional cases (2 and 3) with typical oozing and crusted erythematous lesions not restricted to the intertriginous regions and to the sides of the neck are unequivocal.

The abnormal reactivity can involve the entire skin in Hailey-Hailey disease (Cram, Muller & Winkelmann, 1967; Winkelmann, 1976). It has not yet been clarified which structure of the integument the various triggering factors attack. Surprising is the experience that split skin grafts in formerly affected intertriginous areas remain symptom free for a long period of time (Berger & Lynch, 1971; Bitar & Giroux, 1970; Happle, 1977; Shelley & Randall, 1969; Sonck & Rintala, 1975; Thorne, Hall & Mladick, 1968). Possibly by reduction or removal of sweat glands in intertriginous regions, a decisive factor for the clinical manifestation of the disease is eliminated (Sonck & Rintala, 1975).

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REFERENCES

- BERGER, R.S. & LYNCH, P.J. (1971) Familial benign chronic pemphigus. Surgical treatment and pathogenesis. *Archives of Dermatology*, **104**, 380.
- BITAR, A. & GIROUX, J.M. (1970) Treatment of benign familial pemphigus (Hailey-Hailey) by skin grafting. *British Journal of Dermatology*, **83**, 402.
- CHALET, M.D., GROVER, R. & ACKERMAN, A.B., (1977) Transient acantholytic dermatosis. A re-evaluation. *Archives of Dermatology*, **113**, 431.
- CRAM, D.L., MULLER, S.A. & WINKELMANN, R.K. (1967) Ultraviolet induced acantholysis in familial benign chronic pemphigus *Archives of Dermatology*, **96**, 636.
- GOTTLIEB, S.K. & LUTZNER, M.A. (1970) Hailey-Hailey disease. An electron microscopic study. *Journal of Investigative Dermatology*, **54**, 368.
- GROVER, R.W. (1971) Transient acantholytic dermatosis. Electron microscope study. *Archives of Dermatology*, **104**, 26.
- GSCHNAIT, F. (1973) Pemphigus familiaris chronicus benignus (Hailey-Hailey). *Hautarzt*, **24**, 243.
- HAPPLE, R. (1977) Operative Behandlung des Pemphigus chronicus benignus familiaris Hailey-Hailey. Paper at 31st Meeting of the German Society of Dermatology, Cologne 1977. Abstract: *Zeitschrift für Haut-und Geschlechtskrankheiten*, **52**, 227.
- HERZBERG, J.J. (1955) Pemphigus Gougerot/Hailey-Hailey. *Archiv für klinische und experimentelle Dermatologie*, **202**, 21.
- LEWIS, G.M. (1948) Disseminated bullous dyskeratoid dermatosis (Benign familial pemphigus). *Archives of Dermatology*, **57**, 499.
- LYLES, TH.W., KNOX, J.M. & RICHARDSON, J.B. (1958) Atypical features in familial benign chronic pemphigus. *Archives of Dermatology*, **78**, 446.
- NÜRNBERGER, F. & MÜLLER, G. (1967) Elektronenmikroskopische Untersuchungen über die Acantholyse bei Pemphigus familiaris benignus. *Archiv für klinische und experimentelle Dermatologie*, **228**, 208.
- PALMER, D.D. & PERRY, H.O. (1962) Benign familial chronic pemphigus. *Archives of Dermatology*, **86**, 493.
- PEHAMBERGER, H., GSCHNAIT, F., KONRAD, K. & HOLUBAR, K. (1977) Transient acantholytic dermatosis Grover. *Zeitschrift für Hautkrankheiten*, **52**, 841.
- PIÉRARD, J. & KINT, A. (1969) Pemphigus familial benign chronique (maladie de Hailey-Hailey): Etude au microscope électronique. *Dermatologica*, **139**, 1.
- SHELLEY, W.B. & RANDALL, P. (1969) Surgical eradication of benign pemphigus from the axillae. *Archives of Dermatology*, **100**, 275.
- SONCK, C.E. & RINTALA, A. (1975) Treatment of familial benign chronic pemphigus by skin grafting. *Acta dermato-venereologica*, **55**, 395.
- THORNE, F.L., HALL, J.H. & MLADICK, R.A. (1968) Surgical treatment of familial chronic pemphigus (Hailey-Hailey disease). Report of a case. *Archives of Dermatology*, **98**, 522.

- WILGRAM, F.F., CAULFIELD, J.B. & LEVER, W.F. (1962) An electronmicroscopic study of acantholysis and dyskeratosis in Hailey and Hailey's disease. *Journal of Investigative Dermatology*, **39**, 373.
- WINKELMANN, R.K. (1976) Dermatopathology. *Journal of Investigative Dermatology*, **67**, 188.
- WOLFF, H.H., CHALET, M.D. & ACKERMAN, A.B. (1977) Transitorische akantholytische Dermatose (Grover). *Hautarzt*, **28**, 78.

Epidermolytic hereditary palmoplantar keratoderma

REPORT OF A FAMILY AND TREATMENT WITH AN ORAL AROMATIC RETINOID

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SUMMARY

This study describes a family of 30 people in which 14 members have hereditary epidermolytic palmoplantar keratoderma. Four patients were treated with an oral aromatic retinoid for up to 5 months. They responded in a uniform and dramatic way: 10–14 days after the onset of therapy, the hyperkeratotic horny layer was sequestered in large sheets resulting in normal appearing skin and restoration of normal surface sensitivity. Biopsies revealed that the underlying disorder of keratinization had remained unchanged. Treatment with the retinoid had to be discontinued as the sensitivity and vulnerability restricted normal function of hands and feet.

Epidermolytic hyperkeratosis (Frost & Van Scott, 1966) is a disorder of keratinization with distinctive histopathological (Ackermann, 1970; Frost & Van Scott, 1966; Lapière, 1932; Lapière, 1957) and ultrastructural (Anton-Lamprecht & Schnyder, 1974; Lapière, 1932; Lapière, 1957) characteristics which may be found in genetically transmitted diseases, certain types of naevi and hamartomas (Ackermann, 1970; Gebhart & Kidd, 1973; Plewig & Christophers, 1975; Schnyder, 1970) or, as an accidental feature, in a variety of acquired hyperkeratotic skin conditions (Ackermann, 1970; Plewig & Christophers, 1975). As a genodermatosis, epidermolytic hyperkeratosis in generalized expression presents as bullous ichthyosiform erythroderma (Frost & van Scott, 1966; Lapière, 1932; Lapière, 1957), but localized manifestations may assume the clinical appearance of hereditary linear naevus or hereditary palmoplantar keratoderma. In these localized lesions, the propensity to form bullae is minimal or absent. The epidermolytic variants of linear naevi and palmoplantar keratoderma thus do not differ appreciably from the common orthohyperkeratotic types, and the diagnosis is often made on an incidental basis.

Although estimates of its incidence are a matter of speculation, epidermolytic hereditary palmoplantar keratoderma (EHPPK) appears to be exceedingly rare. Hitherto, only four affected families (Klaus, Weinstein & Frost, 1970; Voerner, 1901) and one single case (Brunsting *et al.*, 1962) have

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been reported in sufficient detail. The first description dates back to 1901 when Voerner reported on a family of 40 members, 16 of whom exhibited a diffuse palmoplantar keratoderma which he thought to be of the common *UNNA-THOST* type (Unna, 1883). Histologically, he found extensive vacuolization of the spinous layer which he attributed to imbibition with sweat. Voerner's family and three others described by Klaus (1970) exhibiting identical clinical and histological features clearly displayed autosomal dominant inheritance with high penetrance. ^3H -thymidine-autoradiography in 2 cases suggested a hyperproliferative nature of the hyperkeratosis in EHPPK (Klaus *et al.*, 1970).

In this study we present a fifth family with EHPPK and report on a therapeutic trial with an oral aromatic retinoid.

CASE REPORTS

The family. The family originates from a secluded valley in the Austrian Alps; most members are farmers or have other manual occupations. At the present time, the family comprises 30 people of three generations, 14 people having EHPPK. Two more family members with the disease have died. The pedigree clearly displays autosomal dominant inheritance (Fig. 1). For this study, 13 members (6 non-affected) were examined.

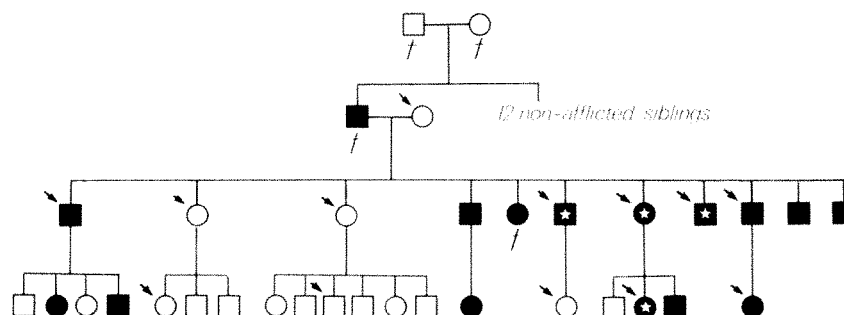


FIGURE 1. Family tree. Arrows: persons examined. Asterisks: persons treated with aromatic retinoid.

Clinical features. All affected persons examined exhibited very similar lesions, which showed little variation as to extent and severity. Palms and soles are covered by thick parchment-like compact hyperkeratotic plates of yellow to brown colour and smooth or slightly scaly surface (Fig. 2a-4). The hyperkeratosis extends to the flexor surfaces of the fingers and toes including the tips. The border to normal skin is sharply demarcated at the wrists and runs a straight course in the thenar and hypothenar regions and the lateral aspects of the fingers, lined by a thin erythematous rim. There is no transgression to the dorsa of the fingers and hand. Finger and toe nails are normal. In the adult proposition ill-defined hyperkeratotic pads overly the knuckles of fingers and toes (Fig. 2c).

Other than their skin condition, all patients appeared healthy. Physical examination, X-ray of skull and chest, as well as otolaryngological ophthalmological and dental examinations did not reveal any associated malformations or illnesses. Routine blood and urine analyses gave normal results. Non-affected members of the family exhibit normal palmoplantar skin without any signs of tylosis. KOH preparations for fungus were negative.

Developmental and functional aspects. All affected patients were born with seemingly normal plantar

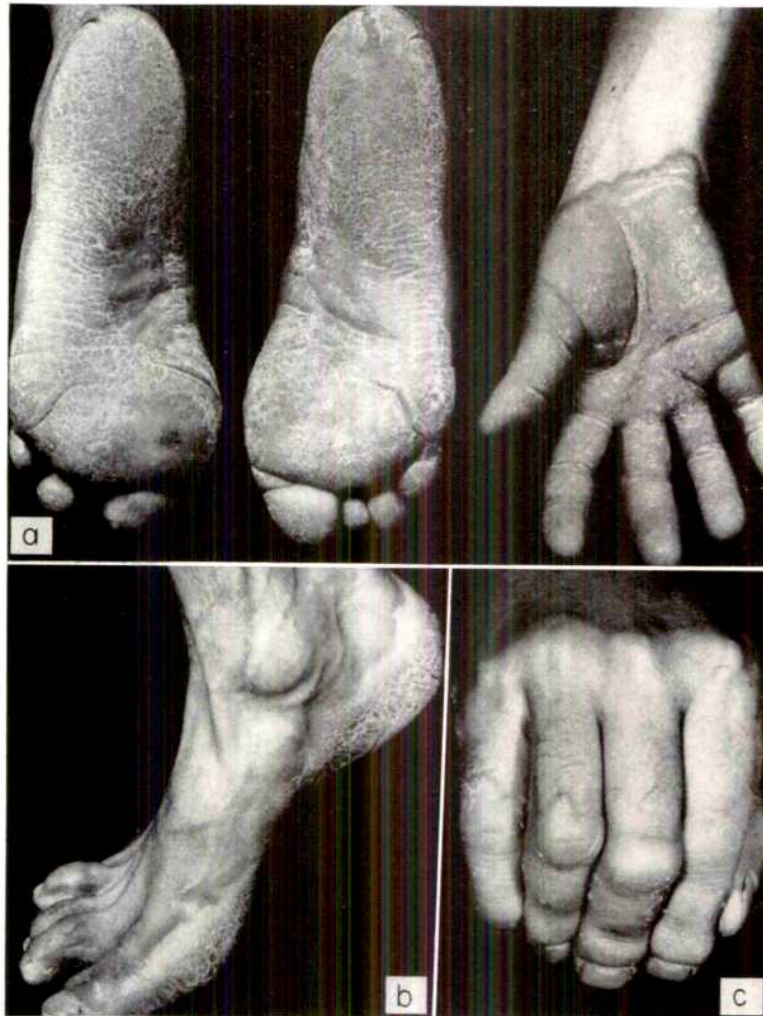


FIGURE 2. Case 1, 34-year-old male. (a and b) Diffuse hyperkeratosis of palms and soles. Note sharp demarcation and erythematous border. (c) Hyperkeratotic pads overlying knuckles.

and palmar skin. With striking regularity, a yellow discoloration and gradual thickening of palms and soles became apparent at the age of three weeks. When the children began to walk, the hyperkeratosis of the soles became more accentuated, full expression being reached at the age of 2 or 3 years. There is some correlation between severity of the lesions and mechanical stress: persons with heavy manual occupations tend to have somewhat more pronounced hyperkeratoses than housewives or people with indoor occupations. Generally, the affected persons are not greatly disturbed by their condition. Each of them had tried all kinds of topical therapy available during childhood but later left the condition untreated for lack of success. Surface and thermal sensitivity is largely abolished. This, however is appreciated by some since they can perform their jobs more carelessly than their normally equipped colleagues. Some disturbance results from hyperhidrosis. There is decreased resistance to mechanical trauma which results in blister formation after forced walking or friction of palmar skin. Detachment



FIGURE 3. Intermediate stages of treatment with aromatic retinoid, 2 weeks after onset of medication—right sole of Case 2 (30-year-old male). Note detachment of large flaps of horny layer.

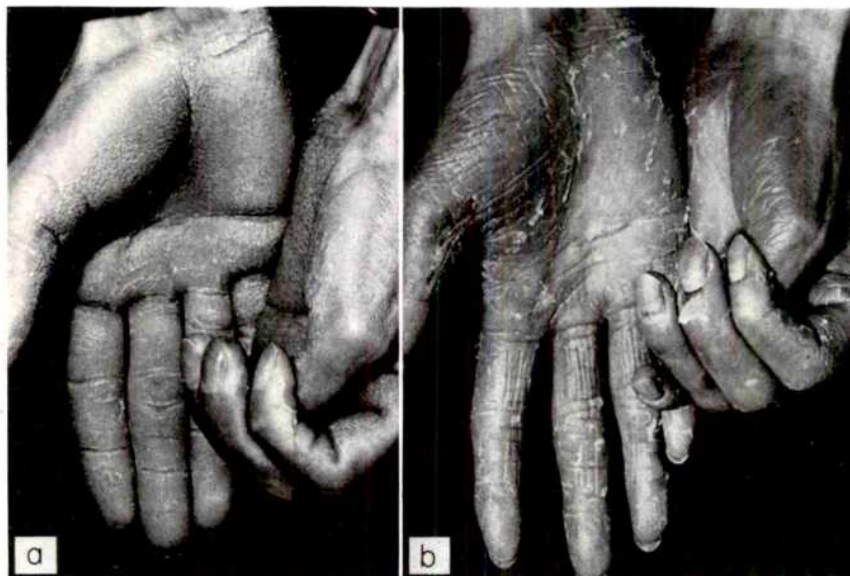


FIGURE 4. Palms of Case 2 before (a), and 4 weeks after (b) onset of treatment with aromatic retinoid. Except for slight scaling and atrophy, the palmar skin appears normal.

of large sheets of hyperkeratotic skin may then occur leading to painful erosions. Also, fissuring of the skin along the palmar creases is not infrequent eventuating in purulent inflammation.

Histopathology (Fig. 5). The epidermis is highly acanthotic. Except for the basal layers, it is highly vacuolized and has a torn and reticulated appearance. Focally, larger cavities are formed by rupturing of cell walls. The nuclei are pyknotic and are absent in more heavily damaged areas. The granular layer is increased to 5 to 10 cell layers, the keratohyalin granules appearing clumpy. The horny layer is compact and orthohyperkeratotic.



FIGURE 5. Histopathology of palmar skin (Case 2): epidermolytic hyperkeratosis. Note sponge-like appearance of upper Malpighian layers by extensive vacuolization (H & E, $\times 250$).

Ultrastructural features (Fig. 6). The spinous cells display numerous cytolytic vacuoles. Keratohyalin granules are huge, clumpy and distorted in a bizarre fashion. Tonofilaments tend to be clumped around the nucleus. There is rarefaction of desmosomes, and the intercellular space is widened. No split desmosomes are present.

Treatment. Four patients (2 males, 34 and 30 years, 1 female, 32 years and a 6-year-old girl) were hospitalized for therapeutic trial with an oral aromatic retinoid (AR)*. AR was given in three to four

* Ethyl all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate (RO 10-9359) kindly supplied by Hoffmann-La Roche Vienna, Austria.

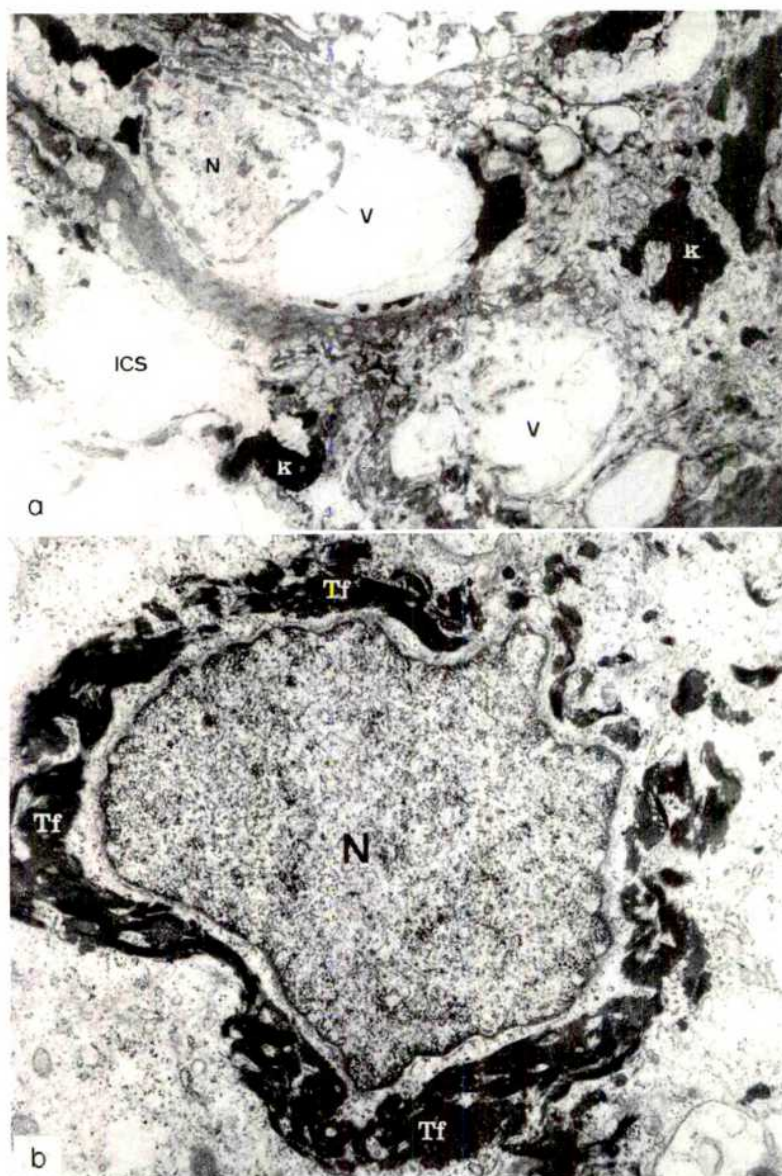


FIGURE 6. Ultrastructure of palmar skin (Case 2). (a) Multiple cytolitic vacuoles (v), widening of intercellular space (ics), bizarre and clumpy keratohyalin (k). (b) Characteristic shell-like aggregation of tonofilaments (Tf) around nucleus (n). (Original magnification: (a) $\times 10,800$, (b) $\times 23,000$).

portions at a total daily dose of 1.5 mg/kg body weight. After 3 weeks, the dose was slowly tapered to 0.3 mg/kg. AR was continued for 3–5 months on an outpatient basis. The following routine laboratory tests were performed at 2–4 week intervals: total blood count, serum transaminases, blood sedimentation rate, fasting blood glucose, prothrombin time and urinalysis. No topical therapy was given except in one male patient who was treated with salicylic acid in white petrolatum.

All patients responded in a strictly reproducible and dramatic way to AR. After a lag period of 10–14 days, the erythematous borders became more accentuated, and the hyperkeratotic plates began to detach along the whole circumference, resembling giant linear blisters. Drainage of these cavities, however, did not produce liquid. Detachment progressed in a roughly centripetal fashion resulting in shedding of huge keratinous flaps (Fig. 3). In one instance, the hyperkeratosis of the soles was sequestered in one single piece. After 1 month, palms and soles exhibited an almost normal appearing, slightly atrophic, reddened and finely scaly skin (Fig. 4). The patients, for the first time in their lives, experienced an excessive amplification of their surface and thermal sensitivity. Biopsies performed in the adult patients revealed substantial reduction of the horny layers but unchanged persistence of epidermolytic hyperkeratosis.

Course. After resuming their normal life and daily work, the patients noticed a largely increased vulnerability of their newly formed skin, which in one case led to severe erosion of the soles. Also, the smooth palmar skin proved a severe handicap in pursuing their farming business. Decrease of AR reduced the vulnerability of the plantar skin to a tolerable degree only at a dose of 0.5 mg/kg body weight, which, however, allowed reestablishment of the hyperkeratotic masses almost as thick as prior to treatment. The cosmetic effect thus being largely abolished, and because of the presence of inconvenient side effects (see below), all patients decided to discontinue AR intake.

Side effects. Throughout the whole period of treatment, the laboratory values remained within normal limits. During the initial treatment phase, all patients complained of dry lips with occasional fissuring and dry rhinitis. These symptoms improved when the dose of AR was reduced. Six to 8 weeks after treatment, all three adult patients developed drowsiness, anorexia and diffuse hair loss which was mild in one and rather severe in 2 cases.

DISCUSSION

This family represents the fifth family described with EHPPK. Clinical symptomatology, course, mode of inheritance and histopathological appearance are congruent with the findings reported (Klaus *et al.*, 1970; Voerner, 1901). Electron microscopy, performed for the first time in EHPPK, revealed an identical ultrastructural pathology as described for epidermolytic hyperkeratosis in bullous ichthyosiform erythroderma (Anton-Lamprecht & Schnyder, 1974; Schnyder, 1970). Obviously, EHPPK is a well defined entity which, however, does not differ appreciably from orthohyperkeratotic diffuse hereditary palmoplantar keratoderma on clinical grounds except for some tendency to develop erosions as a consequence to mechanical stress.

Our patients treated with AR represent an instructive example of the effectiveness as well as of the limitations of this novel therapeutic modality. Oral aromatic retinoids have emerged as a major achievement in the therapy of hyperkeratotic skin conditions including such heterogeneous disorders as psoriasis (Fritsch *et al.*, 1978; Ott, 1977), lichen planus, bullous ichthyosiform erythroderma (Schorr & Papa, 1973), lamellar ichthyosis, erythrokeratoderma figurata variabilis (Van der Wateren & Cormane, 1977), Darier's disease (Hönigsmann, Fritsch & Jaschke, 1978), leukoplakias, oral florid papillomatosis, actinic keratoses and even common warts. Obviously, there is hardly a common denominator in the pathogenesis of these conditions which might be linked to a specific property of the retinoids. At the present time their mode of action is only poorly understood; moreover, being powerful stimulators of DNA-synthesis, their beneficial effect on hyperproliferative skin conditions appears paradoxical.

In our patients, AR was the first therapy regimen ever employed which was capable of completely removing the hyperkeratotic palmoplantar pads and thus to restore an unimpaired perception of

surface sensitivity. Despite its dramatic clinical effect, the underlying disturbance of keratinization remained unchanged, and the apparently normal skin produced by AR proved functionally inadequate compelling the patients to prefer function to aesthetics and to discontinue intake of AR. Similarly, Schnyder & Gloor (1977) noted persistence of epidermolytic hyperkeratosis in patients with bullous ichthyosiform erythroderma treated with the aromatic retinoid, accompanied by an increased tendency to form bullae. Improvement of the epidermolytic changes following topical retinoic acid therapy, however, has been claimed (Schorr & Papa, 1973). Obviously, the effect of AR in EHPPK is largely symptomatic, and it may be expected to be similarly symptomatic in most other hyperkeratotic conditions. In a given hyperkeratotic disorder, therefore, a beneficial effect of AR depends largely on the functional quality of the diseased skin and the degree of mechanical trauma to which the diseased regions are exposed. Disorders with an inherent propensity to form erosions e.g. EHPPK or pachyonychia congenita (unpublished observation), thus arrive at an even less functionally satisfactory state after thinning of the hyperkeratosis whereas orthohyperkeratotic palmoplantar keratoderma, both of the diffuse and punctate variety, are considerably improved after AR-therapy (unpublished observation). Therefore, despite its efficacy in a broad spectrum of hyperkeratotic disorders, the benefit achieved with oral AR therapy is linked to the nature of the underlying condition.

REFERENCES

- ACKERMANN, B.A. (1970) Histopathologic concept of epidermolytic hyperkeratosis. *Archives of Dermatology*, **102**, 253.
- ANTON-LAMPRECHT, I. & SCHNYDER, U.W. (1974) Ultrastructure of inborn errors of metabolism. VI. Inherited ichthyoses—a model system for heterogenetics in keratinization disturbances. *Archiv für Dermatologische Forschung*, **250**, 207.
- BRUNSTING, L.A., KIERLAND, R.R., PERRY, O. & WINKELMANN, R.K. (1962) Epidermolysis bullosa versus keratoderma palmare et plantare. *Archives of Dermatology*, **85**, 676.
- FRITSCH, P.O., HÖNIGSMANN, H., JASCHKE, E. & WOLFF, K. (1978) Augmentation of oral methoxsalen-photochemotherapy with an oral retinoic acid derivative. *Journal of Investigative Dermatology*, **70**, 178.
- FROST, P. & VAN SCOTT, E.J. (1966) Ichthyosiform dermatoses: classification based on anatomic and biometric observations. *Archives of Dermatology*, **94**, 113.
- GEBHART, W. & KIDD, R.L. (1973) Das solitäre epidermolytische Akanthom. *Zeitschrift für Hautkrankheiten*, **47**, 1.
- HÖNIGSMANN, H., FRITSCH, P.O. & JASCHKE, E. (1978) Hypertroph verhornende Variante des Morbus Darier: erfolgreiche Behandlung mit einem aromatischen Retinoid (Ro 10-9359). *Hautarzt*, in press.
- KLAUS, S.N., WEINSTEIN, G.D. & FROST, P. (1970) Localized epidermolytic hyperkeratosis. A form of keratoderma of the palms and soles. *Archives of Dermatology*, **101**, 272.
- LAPIÈRE, S. (1932) Epidermolyse ichthyosiforme congénitale (érythrodermie ichthyosiforme congénitale forme bulleuse de Brocq). *Annales de Dermatologie*, **3**, 401.
- LAPIÈRE, S. (1957) L'érythrodermie ichthyosiforme congénitale bulleuse letale. *Annales de Dermatologie*, **84**, 5.
- PLEWIG, G. & CHRISTOPHERS, E. (1975) Nevroid follicular epidermolytic hyperkeratosis. *Archives of Dermatology*, **111**, 223.
- OTT, F. (1977) Behandlung der Psoriasis mit einem oral wirksamen aromatischen Retinoid. *Schweizer Medizinische Wochenschrift*, **107**, 144.
- SCHNYDER, U. (1970) Inherited ichthyoses. *Archives of Dermatology*, **102**, 240.
- SCHNYDER, U.W. & GLOOR, M. (1977) Bullöse Erythrodermie congénitale ichthyosiforme (Fall 20). Gemeinschaftstagung der Südwestdeutschen Dermatologen-Vereinigung und der Vereinigung Rheinisch-Westfälischer Dermatologen in Heidelberg vom 8.–10. Oktober 1976. *Zeitschrift für Hautkrankheiten*, **52**, 809.
- SCHORR, W.F. & PAPA, C.M. (1973) Epidermolytic hyperkeratosis. Effect of tretinoin therapy on the clinical course and the basic defects in the stratum corneum. *Archives of Dermatology*, **107**, 556.
- UNNA, P.G. (1883) Über das Keratoma palmare et plantare hereditarium. *Archiv für Dermatologie und Syphilis*, **15**, 231.
- VAN DE WATEREN, A.R. & CORMANE, R.H. (1977) Oral retinoic acid as therapy for erythrokeratoderma variabilis. *British Journal of Dermatology*, **97**, 83.
- VOERNER, H. (1901) Zur Kenntnis des Keratoma hereditarium palmare et plantare. *Archiv für Dermatologie und Syphilis*, **56**, 3.

Buckley's syndrome

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SUMMARY

An infant girl with chronic eczema, recurrent infections, elevated IgE and impaired neutrophil chemotaxis appeared to belong to the group of patients described by Buckley, Wray & Belmaker (1972). An hereditary influence is suggested by a similar defect found in the patient's mother. Levamisole improved the *in vitro* defect and the clinical picture.

The case of a child with Buckley's syndrome is presented which appears to be the first to be described in Europe.

CASE REPORT

A 2½-year-old female infant, with recurrent eczematous dermatitis, developed some vesicles affecting the face and nape of the neck on the 30th day of life and was treated with steroid creams, but relapsed several times. In the 60 subsequent days, an abscess in the submandibular area and on the scalp needed incision and drainage. She also had tonsillitis and otitis.

Physical examination revealed a normal child with an erythematous and vesiculo-pustular dermatitis on the nape, buttocks and knees resembling atopic dermatitis, and a rectovaginal fistula that apparently had not been preceded by abscesses.

Since the immunological studies had demonstrated an impaired chemotaxis, 4 courses of different length of levamisole, 25 mg daily, were administered with 7 day intervals (Fig. 1).

Six days after the end of the first levamisole course, 2 further abscesses appeared on the buttocks accompanied by high fever that required oral administration of erythromycin, 400 mg/day. The fever rapidly ceased and the abscesses cleared. Over the past 12 months only one further abscess has occurred.

METHODS

Neutrophil chemotaxis and random migration were determined using casein 5 mg/ml (Hammersten, Merck) as a chemotactic stimulus and 3 µm filters (Millipore) (Wilkinson, 1974).

Adhesiveness was studied by the nylon fibres method (McGregor, Spagnuolo & Lentnek, 1974)

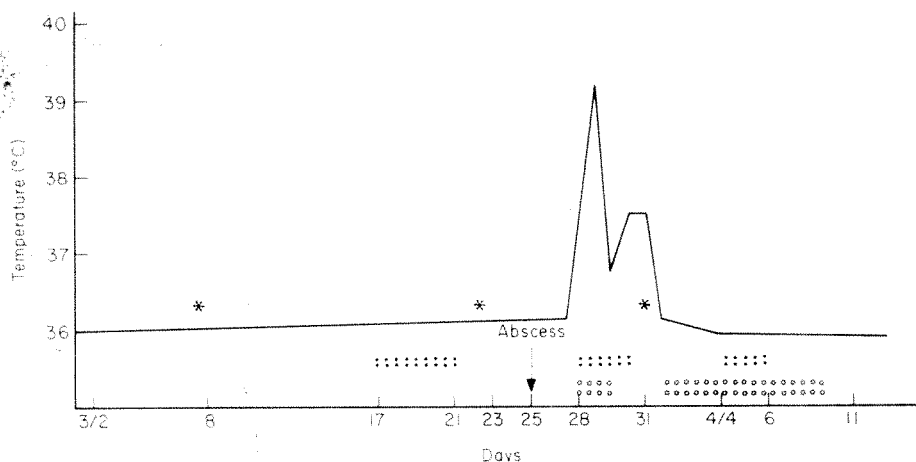


FIGURE 1. * PMN function assays. (●) Levamisole 25 mg/day. (○) Erythromycin 400 mg/day.

and phagocytosis with $0.81 \mu\text{m}$ polystyrene particles (Labtex, Difco) (Chrétien & Garagusi, 1972).

The spontaneous nitro-blue-tetrazolium (NBT) reduction test was performed as described by Park, Fikring & Smithwick (1968), and the endotoxin-stimulated NBT reduction test was done (Park & Good, 1970).

Lymphocyte function tests were performed on cells isolated on a Hypaque-Ficoll gradient (Boyum, 1968). T and B lymphocytes were counted with the E and EAC rosette tests (Jondal, Holm & Wigzell, 1972).

Cell mediated immunity (CMI) was investigated *in vitro* through the lymphocyte transformation response using phytohaemagglutinin, PPD, concanavalin A and pokeweed mitogen as stimulating agents (Ling & Kay, 1975).

Ig G, Ig A, Ig M and C3-C4 components of complement were estimated by radial immunodiffusion on Behringwerke plates. Ig E was measured by radioimmunoassay (Pharmacia, Uppsala).

Candida albicans was cultivated on Sabouraud Dextrose Agar (BBL) supplemented with chloramphenicol (0.4 mg/ml). *Staphylococcus aureus* was isolated on Mannitol Salt Agar (Difco) and then identified with the coagulase test.

RESULTS

The results of the immunological studies, before and after the levamisole treatment, are collected in Tables 1 and 2. We wish to stress (i) the raised level of IgE (900 IU/ml) (normal values up to 190 IU/ml); (ii) the reduction to 40% of neutrophil chemotaxis and its dramatic improvement to 100% after the first levamisole course; (iii) the absence of the spontaneous NBT reduction and a diminution of the stimulated NBT test (13.5%), and their improvement after the levamisole treatment.

In addition, *C. albicans* was cultivated from faeces ($240,000/\text{g}$), mouth, and forearm skin. *Staph. aureus* also was cultivated from the abscesses and from normal skin.

COMMENT

The main components of Buckley's syndrome appear to be: chronic eczematous dermatitis, recurrent abscesses, elevated serum Ig E, and defective neutrophil chemotaxis. Peripheral blood eosinophilia,

TABLE 1

Tests	Patient				Mother 26-27/4	Father 26/4	Normal range
	Before treatment 8-9/3	After treatment					
		23-24/3	31/3-1/4	5/5			
Leucocytes	13200	11600	10600	10200	7400	5200	5000-15000†
Eosinophils	528	n.d.	1378	510	296	52	20-500†
PMN functions							
Random migration	100	100	n.d.	100	100	100	90-110
Chemotaxis‡	40	100	n.d.	100	75	100	90-110
Adhesiveness	73	87	n.d.	n.d.	81	86	> 75
Phagocytosis	49	45	44	47	41	41	> 40
Spont. NBT red.	0*	1	1	2	0.25	1	≤ 7
Stim. NBT red.	13.5*	37	44	20	10	30	> 20
Alkal. phosph. score	87	n.d.	n.d.	n.d.	40	52	20-100
Peroxidase score	185	n.d.	n.d.	n.d.	191	205	150-250

Average of 2 determinations in 2 successive days.

* Tested in a single day only.

† For children.

‡ Expressed by the measurement of the distance travelled by the leading front (Zigmoid & Hirsch, 1973) as a percentage of normal control.

TABLE 2

Tests	Patient		Range of normality for patient's age
	Before treatment	After treatment	
E rosettes	42%	48%	50-70%
EAC rosettes	27%	25%	20-30%
PHA stim. index	35	30.1	> 80
Con A stim. index	3.4	4.4	> 20
IgG mg/100 ml	700	n.d.	892 ± 183
IgM mg/100 ml	120	n.d.	61 ± 19
IgA mg/100 ml	35	n.d.	71 ± 37
IgE IU/ml	900	900	< 190
C ₃ mg/100 ml	62	n.d.	55-120
C ₄ mg/100 ml	27	n.d.	20-40

n.d. = not done.

lung infections, urticaria, and *C. albicans* in the mouth and on the skin seem to be incidental findings sometimes reported (Hill & Quie, 1974; Dahl, Green & Quie, 1976).

This syndrome should be distinguished from Job's syndrome (Davis, Schaller & Wedgwood, 1966) and from chronic mucocutaneous candidiasis (CMCC) (Van Scoy *et al.*, 1975).

In Job's syndrome abscesses are 'cold', whilst our patient's are typical Staphylococcal boils. Moreover our case did not have red hair (3/4 of the cases in Hill *et al.* series, 1974) and atrophic

fingerails (1/2 in the original Davis *et al.* paper, 1966) which are inconstantly found in Job's syndrome.

CMCC (Quie & Chilgren, 1971) cannot be confused unless it is associated with eczema and recurrent infections; in such few cases the concomitant elevated IgE level and chemotactic neutrophil defect suggest that they are a mere overlap of the two disorders (Hill & Quie, 1975).

Our patient therefore is similar to those of Buckley *et al.* (1972), showing most of their features save urticaria and lung infections; a peculiar finding in our case is the partial defect in the stimulated NBT test, which could be related to a faulty bactericidal activity.

As in similar cases (Van Scoy *et al.*, 1975; Edelson *et al.*, 1973), the modest impairment of chemotaxis and stimulated NBT test found in the patient's mother (Table 1) suggested a familial pattern.

Levamisole seems to have been helpful, probably stimulating chemotaxis and NBT reduction (Wright, Kirkpatrick & Gallin, 1977; Patrone, Dallegri & Sacchetti, 1978) even if the associated course of erythromycin (Fig. 1) casts some doubt on its real effectiveness.

REFERENCES

- BOYUM, A. (1968) Separation of leukocytes from blood and bone marrow. *Scandinavian Journal of Clinical and Laboratory Investigation*, **21**, Suppl. 97.
- BUCKLEY, R.H., WRAY, B.B. & BELMAKER, E.Z. (1972) Extreme hyperimmunoglobulinemia and undue susceptibility to infection. *Pediatrics*, **49**, 59.
- CHRETIEN, J.H. & GARACUSI, V.F. (1972) Corticosteroid effect on phagocytosis and NBT reduction by human polymorphonuclear neutrophils. *Journal of the Reticuloendothelial Society*, **11**, 358.
- DAHL, M.V., GREENE, W.H. & QUIE, P.G. (1976) Infection, dermatitis, increased IgE and impaired neutrophil chemotaxis. *Archives of Dermatology*, **112**, 1387.
- DAVIS, S.D., SCHALLER, J. & WEDGWOOD, R.J. (1966) Job's syndrome. Recurrent 'cold' staphylococcal abscesses. *Lancet*, **i**, 1013.
- EDELSON, P.J., STITES, D.P., GOLD, S. & FUDENBERG, H.H. (1973) Disorders of neutrophil function. Defects in the early stages of the phagocytic process. *Clinical and Experimental Immunology*, **13**, 21.
- HILL, R.H. & QUIE, P.G. (1974) Raised serum IgE levels and defective neutrophil chemotaxis in three children with eczema and recurrent bacterial infections. *Lancet*, **i**, 183.
- HILL, R.H. & QUIE, P.G. (1975) Defective neutrophil chemotaxis associated with hyperimmunoglobulinemia E. In: *The Phagocytic Cell in Host Resistance*. (Ed. by J.A. Bellanti, and D.H. Dayton) Raven Press, New York.
- JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes. I. A large proportion of lymphocytes forming non-immune rosettes with sheep red blood cells. *Journal of Experimental Medicine*, **136**, 207.
- LING, N.R. & KAY, J.E. (1975) *Lymphocyte Stimulation*. North Holland, Amsterdam, Oxford.
- MCGREGOR, R.R., SPAGNUOLO, P.J. & LENTNEK, A.L. (1974) Inhibition of granulocyte adherence by ethanol, prednisone and aspirin measured with a new assay system. *New England Journal of Medicine*, **271**, 642.
- PARK, B.H., FIKRING, S.M. & SMITHWICK, E.M. (1968) Infection and nitro-blue-tetrazolium reduction by neutrophils. A diagnostic aid. *Lancet*, **ii**, 532.
- PARK, B.H. & GOOD, R.A. (1970) NBT test stimulated. *Lancet*, **ii**, 616.
- PATRONE, F., DALLEGRI, F. & SACCHETTI, C. (1978) Defective neutrophil chemotaxis corrected by levamisole in Crohn's disease. Case report. *Acta hepato-gastroenterologica*, in press.
- QUIE, P.G. & CHILGREN, R.A. (1971) Acute disseminated and chronic mucocutaneous candidiasis. *Seminars in Haematology*, **8**, 227.
- VAN SCOY, R.E., HILL, R.H., RITTS, R.E. & QUIE, P.G. (1975) Familial neutrophil chemotaxis defect, recurrent bacterial infections, mucocutaneous candidiasis and hyperimmunoglobulinemia E. *Annals of Internal Medicine*, **82**, 766.
- WILKINSON, P.C. (1974) *Chemotaxis and Inflammation*. Churchill, Edinburgh & London.
- WRIGHT, D.G., KIRKPATRICK, C.H. & GALLIN, J.I. (1977) Effects of levamisole on neutrophils and mononuclear cells from normal individuals and from patients with abnormal leukocyte chemotaxis. In: *Control of Neoplasia by Modulation of the Immune System* (Ed. by M.A. Chirigos *et al.*). Raven Press, New York.
- ZIGMOND, S.H. & HIRSCH, J.G. (1973) Leukocyte locomotion and chemotaxis. New methods for evaluation and demonstration of cell-derived chemotactic factor. *Journal of Experimental Medicine*, **137**, 387.

Localized crusting as an artefact

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SUMMARY

Four patients are reported, two with gross crusting of the nose and two with gross crusting of the lips considered to be artefacts. The crusting was easily removed using soft paraffin or Viscopaste.

Case 1

A female aged 28. She gave a 4-year history of crusting of the nose (Fig. 1). She had not left the house for 9 months. She had been married for a short time but was now divorced. The crusting started during the break up of her marriage. She admitted to a 'slight feeling of relief' when a recent boy friend finally abandoned her.

Previously she had consulted an E.N.T. surgeon who reported 'I can find nothing really much wrong with this patient's nose except she seems to be rubbing it a lot'. She had also consulted a plastic surgeon who wrote 'feels run down, living alone'. The crusting could be removed easily using Viscopaste bandage. She refused to have a biopsy.

A psychiatrist described her as 'insecure and rather dependent on her parents'.

Case 2

A female in her early twenties, almost a twin of Case 1 except she was even less cooperative. She refused to be photographed, or to consult a general physician or psychiatrist.

Case 3

A female aged 18. For 9 months she had suffered from crusting of the lips (Fig. 2). Her own doctor described her as 'a rather strange girl who is extremely reluctant to see anyone and would prefer just to sit at home'.

The lips showed gross crusting which could be removed easily using soft paraffin.

Case 4

A female aged 53 with a 9-month history of crusting of the lips. She was described as 'a nervous, tiresome sort of patient' who lives alone and who is unwilling to remove the crust using simple treatment. She would prefer the District Nurse to visit daily to treat the lips.

When first examined the lips were thickly crusted and, as she lived in an isolated village with a poor bus service, she was admitted to hospital. Within a few days the crusts were removed, using soft paraffin, leaving normal looking lips. On discharge, within 3 weeks there was a return of her original state.

She refused to consult a psychiatrist.



FIGURE 1. Case 1



FIGURE 2. Case 3

DISCUSSION

The condition is rare. Only four cases have been seen in 24 years.

In two of these four patients the lips were affected and the differential diagnosis of exfoliative cheilitis had to be considered. Exfoliative cheilitis, however, is a chronic superficial inflammatory disorder of the vermilion borders of the lips characterized by persistent scaling. Whereas, the patients described showed thick crusting of the lips and when the crusts were removed, which was easily done, the underlying lips were normal.

Candida was not found in any of these patients.

Artefacts vary widely in their morphology. The method employed by these patients was to pick at the skin of the nose and lips with their finger nails.

PSYCHIATRIC OPINION (DR E. HOWARTH)

The patients are female, insecure, timid and dependent on their parents. They rarely leave their homes, preferring to sit at home rubbing and picking at their nose or lips.

The patients are malingering in order to opt out of their ordinary occupational or social responsibilities. It is usually difficult to get them to consider their problem in psychological terms or to get them to keep a psychiatric appointment.

Eruptive melanocytic naevi following severe bullous disease

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SUMMARY

Two patients developed eruptive naevi following severe bullous disease. The naevi developed after an interval of months and remain unchanged 4 years later. Eruptive naevi have been described after convulsions and both patients developed bullous disease after treatment for convulsions.

Melanocytic naevi are the most common tumour of the skin, and range between 20–40 per person. Their numbers increase through childhood, reaching a peak during puberty and adolescence; later in life they may involute. Pregnancy, corticosteroid or oestrogen therapy may produce crops of naevi (Walton, 1971). Lentigines have an increased number of melanocytes but no naevus cells and may in some cases be the precursors of melanocytic naevi. In the multiple lentiginosis syndrome, mental retardation and convulsions have been described (Polani & Moynahan, 1972). Two patients are described who developed many eruptive naevi months after recovering from severe widespread bullous disease.

Case 1

In August 1973, the patient, a girl who was aged 9 years, had a blackout during micturition and was prescribed phenobarbitone. Twelve days later she developed a widespread morbilliform eruption followed by the appearance of bullae. Other lesions were the more typical target or iris lesions of erythema multiforme. Ulcers appeared in her mouth, her lips and eyes were swollen and closed by muco-purulent crusts, and the vulva became reddened (Fig. 1).

During her severe illness, which lasted for 3 weeks, she was treated with prednisolone, initially in doses of up to 60 mg daily. Virus and bacteriological studies yielded no further information, and she had taken no other drugs. The diagnosis was Stevens–Johnson syndrome caused by phenobarbitone.

In February 1974, hyperpigmentation was noted over her trunk and axillae, together with hyperkeratosis around the nipples and hairline. Small pigmented maculo-papular lesions were seen extending over the mid sternal region, and some finger and toe nails were dystrophic (Fig. 2). Blepharoconjunctivitis was also noted, and she has been treated since that time for trichiasis. She remains a normal healthy child, and has suffered no further convulsions.



FIGURE 1. Case 1. Acute bullous lesions of erythema multiforme.

Biopsies from the chest lesions in 1974 showed nests of naevus cells at the junctional zone of the epidermis, and the early development of a compound naevus (Fig. 3).

In 1977, at the age of 14 years, the naevi show no increase in number, size or distribution; the hyperkeratotic skin over the nipples has persisted. A further biopsy again showed early pigmented cellular naevi.

Case 2

A boy was 8 years of age in August 1969, when he developed petit-mal, and was started on phenobarbitone. Several weeks later, in mid-September, phenytoin was added to his anti-convulsant regime. In October he was admitted to hospital with a 24 h history of sore eyes, and widespread erythema over his face, limbs and trunk. Bullae developed over most of his body surface, and his mouth and eyes became crusted and swollen. A diagnosis of toxic epidermal necrolysis was made. Bacteriological and virus studies produced no obvious pathogen. The condition was thought to be drug induced, with phenobarbitone or phenytoin as the agent. He was treated with erythromycin by mouth, chloramphenicol eyedrops and Terracortril ointment, and was discharged 3 weeks later.

In February 1973, pigmented macular lesions which had been present for more than 6 months were noted on his face, neck, back and arms. He had continued to suffer frequent convulsions with myoclonic jerks in the mornings, generalized tonic attacks most evenings with occasional automatisms and atonic convulsions.

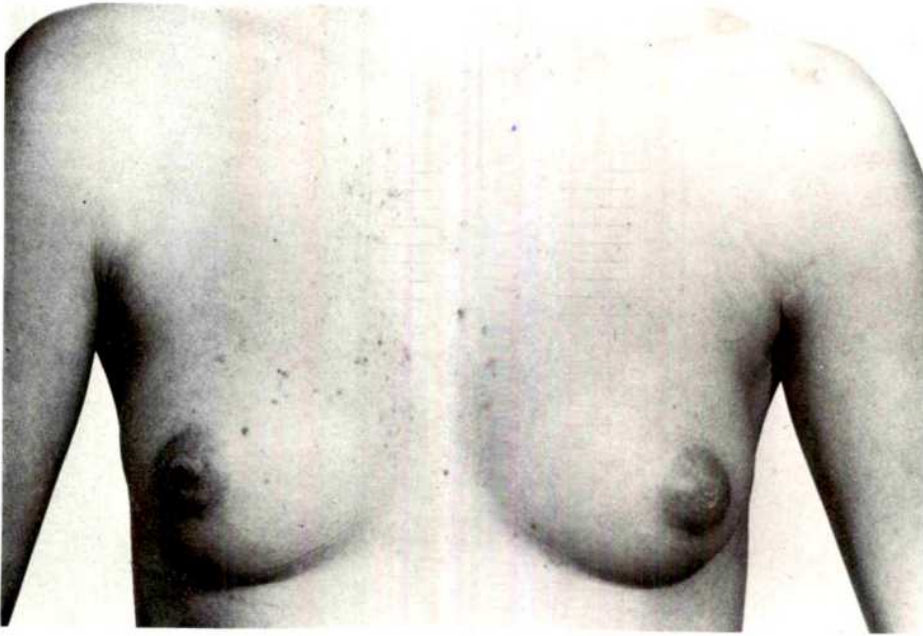


FIGURE 2. Case 1. Melanocytic naevi over chest and dyskeratosis over nipples.

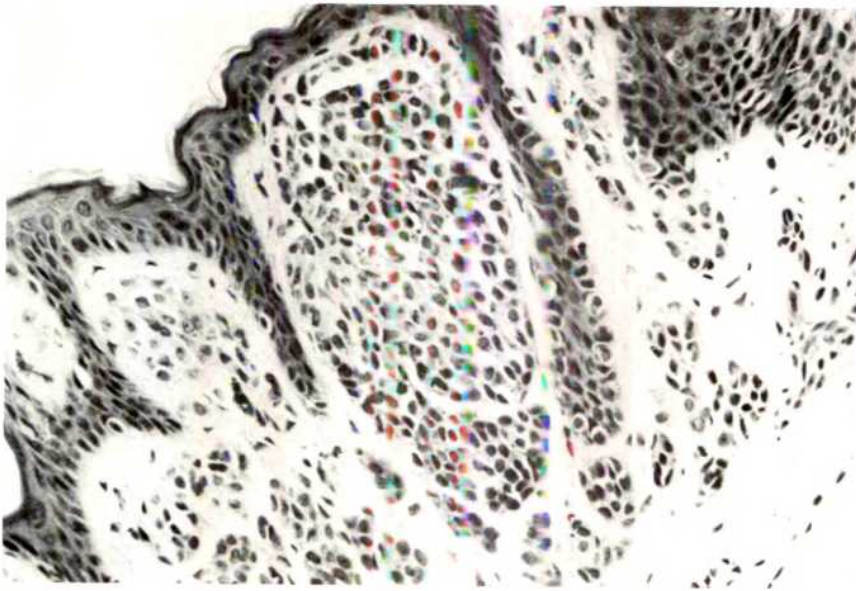


FIGURE 3. Case 1. Compound naevus.



FIGURE 4. Case 2. Grouped naevi over scapulae and lumbar region.



FIGURE 5. Case 2. Early melanocytic naevus.

Treatment was continued with Mysoline, Epilim and Valium. No intellectual advancement had been made since the age of 8 years, and continued treatment for blepharo-conjunctivitis has been necessary.

A biopsy from his skin in October 1974 showed elongated rete ridges, with nests of naevus cells stripping off from the basal layer.

In 1977, his clinical appearance was unchanged; many clusters of flat melanocytic naevi were noted over his back, neck and upper limbs (Fig. 4).

A further biopsy in October 1977 showed increased melanocytic activity in the basal layer, particularly at the tips of the rete ridges, where sequestered nests of naevus cells were seen. The changes were those of early pigmented cellular naevi (Fig. 5).

DISCUSSION

In 1977 Kopf *et al.* described two children who developed widespread eruptive naevi after severe bullous disease. Clinical details of treatment were not available in the report, but both children were aged 8 years at the onset of their disease. Diagnoses of toxic epidermal necrolysis and Stevens-Johnson syndrome were made. The boy described in their article developed measles followed by the Stevens-Johnson syndrome, and during the illness he lost his hair and nails. Six to eight months later multiple pigmented lesions were noted on his trunk in areas involved by the primary disease. Many naevi developed on his skin within a few months. Five years later corneal scarring, nail dystrophy and eruptive naevi over large areas of his back were noted. The second American case was an 8-year-old girl who had toxic epidermal necrolysis. She developed hyperpigmentation 6-8 months later over her trunk, together with many naevi on her upper arms and right axilla. Four years later her naevi remain unchanged. Histology from both cases showed nests of naevus cells at the dermo-epidermal junction. Some cells had migrated into the dermis to produce compound naevi.

No alteration in the clinical appearance or histology was seen in the American cases or our own after a comparable follow-up period.

The hypothesis offered by Kopf *et al.* was that in the process of re-epithelialization melanocytic control had become disorganized, but in many comparable situations, e.g. burns, this does not produce naevi. Our patients differ slightly in that they both were treated for a cerebral disturbance with anti-convulsive therapy prior to the appearance of their naevi. The girl has had no further neurological episodes and remains otherwise normal. The boy who is now 16 years old is severely mentally handicapped and has made no intellectual headway since his primary illness. Intensive anticonvulsant therapy is required for his poorly controlled epileptic attacks.

What other factors may be relevant in the production of naevi? Corticosteroid therapy was thought to produce crops of naevi (Walton, 1971). Only the girl, who has far fewer naevi than the boy, was given systemic corticosteroids during her illness. What is the role of endogenous steroids? Are the convulsive episodes and the naevi related? Both naevus cells and the Schwann cells of peripheral nerves as well as spinal ganglia and glial elements derive from a common source within the neural crest (Boyd, 1961).

Sanderson (1960) described the onset of cellular naevi in a young man following a transient loss of consciousness. Capetanakis (1975) reported a patient who 6 years after producing crops of eruptive naevi developed frequent epileptic attacks.

Lentiginosities may be the forerunners of melanocytic naevi in some cases and both mental retardation and convulsions have been reported in association with lentiginosis (Polani & Moynahan, 1972; MacMillan, 1969).

Drug induced toxic epidermal necrolysis and erythema multiforme may involve the basal layer of

the epidermis (Pearson, 1977). The scalded skin syndrome in children, produced by a staphylococcal epidermolytic toxin, produces cleavage at a higher plane within the epidermis. If a model could be found using a strain of laboratory animal known to pigment and in which basal layer disruption could readily be produced, then further progress in the understanding of these four cases in children might be made. We might also learn more of the pathogenesis of melanocytic naevi in general.

ACKNOWLEDGMENT

Dr C.M.Ridley kindly provided information and photographs for the girl Case 1.

REFERENCES

- BOYD, J.D. (1961) In: *Progress in the Biological Sciences in Relation to Dermatology* (Ed. by A.Rook), p. 3. Cambridge University Press.
- CAPETANAKIS, J. (1975) Juvenile melanoma disseminatum. *British Journal of Dermatology*, **92**, 207.
- KOPF, A.W., GRUPPER, C., BAER, R.L. & MITCHELL, J.C. (1977) Eruptive naevocytic naevi after severe bullous disease. *Archives of Dermatology*, **113**, 1080.
- MACMILLAN, D.C. (1969) Profuse Lentiginosis, mild cardiac abnormality and small stature. *Proceedings of the Royal Society of Medicine*, **62**, 1011.
- PEARSON, R.W. (1977) Advances in the diagnosis and treatment of blistering diseases. A selective review. In: *Year Book of Dermatology*, (Ed. by F.D. Malkinson and R.W. Pearson), p. 38. Year Book Publishers, Chicago.
- POLANI, P.E. & MOYNAHAN, E.J. (1972) Progressive cardiomyopathic lentiginosis. *The Quarterly Journal of Medicine*, **41**, 205.
- SANDERSON, K.V. (1960) Eruptive telangiectatic cellular naevi. *British Journal of Dermatology*, **92**, 207.
- WALTON, R.G. (1971) *Pediatric clinics of N. America*. 18. No. 3, p. 899.

Brief Communication

Sodium cromoglycate in the treatment of dermatitis herpetiformis

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Several papers have been published on the effectiveness of oral sodium cromoglycate in the prevention of the intestinal and cutaneous manifestations of food allergies (Bascmba *et al.*, 1977; Eade & Wright, 1976; Esteban, 1977; Kingsley, 1974; Vaz, 1978).

Thinking that it might help patients with dermatitis herpetiformis, because of their allergy to gluten, oral sodium cromoglycate was given to fifteen patients with this condition in a dosage of 1 g/day in divided doses 10 min before food. The patients were told to empty the capsules into half a tumbler of warm water and take the contents in liquid.

All the patients had dermatitis herpetiformis proved by skin biopsy and all were controlled fairly well by dapsone.

Although the response to sodium cromoglycate was not immediate, taking about a month before improvement was noticed, four out of the fifteen patients were able to stop dapsone completely and to eat gluten-containing foods without any cutaneous manifestations. All these four patients have now been without dapsone for 9 months with no relapse, relying entirely on the sodium cromoglycate and all say that they have never felt better.

TABLE 1. Response to sodium cromoglycate in dermatitis herpetiformis patients

No improvement	Improved, taking less dapsone	Cleared completely
2	9	4 All taking gluten, 3 remain clear after 9 months on SCG alone

Two of the fifteen patients received no benefit at all from the sodium cromoglycate but the remaining eleven were all able to lower their dose of dapsone without blistering or itching. There were no side effects.

Sodium cromoglycate is thought to act in asthma by stabilising mast cell membranes and by preventing, not the reaction between antigen and antibody, but the subsequent release of the mediators which give rise to the hypersensitivity symptoms. Pepys *et al.* (1968), however, have shown that it interferes not only with Type I allergic reactions but also with Type III (Arthus) reactions.

It has been found, in our laboratory, that sodium cromoglycate will inhibit the release of the lysosomal enzyme B Glucuronidase from stimulated neutrophils, as corticosteroids also do (to be published). Its action, therefore, may be that of a membrane stabiliser preventing the release of enzymes from neutrophils when they are in contact with immune complexes which are known to exist in this condition.

From the patient's point of view it is inconvenient to have to take capsules before every meal, especially when they have to be dissolved in water, instead of one or two tablets a day. But if sodium cromoglycate will free some of these patients from the restrictions of a gluten free diet, such disadvantages may be considered minor. What effect this substance has on the gut in this disease will obviously have to be investigated.

ACKNOWLEDGMENTS

I would like to thank Fison's Pharmaceuticals Ltd. for supplying the sodium cromoglycate (Intal).

REFERENCES

- BASOMBA, A., CAMPOS, A., VILLALMANZO, I.G. & PELAEZ, A. (1977) The effect of sodium cromoglycate in patients with food allergies. *Acta Allergologica, Suppl.* **13**, 96.
- EADE, O.E. & WRIGHT, R. (1976) Dietary hypersensitivity. The gastroenterologists point of view. *Journal of Human Nutrition*, **30**, 157.
- ESTEBAN, M.M., OJEDA, J.A., LASO, M.T. & PASCUAL, C. (1977) Oral sodium cromoglycate in food allergy. *Acta Allergologica Suppl.* **13**, 102.
- KINGSLEY, P.J. (1974) Oral sodium cromoglycate in gastro-intestinal allergy. *Lancet*, **ii**, 1011.
- PEPYS, J., HARGREAVE, F.E., CHAN, M. & MCCARTHY, D.S. (1968) *Lancet*, **ii**, 134.
- VAZ, G.A., TAN, L.K.T. & GERRARD, J.W. (1978) Oral cromoglycate in the treatment of adverse reactions to foods. *Lancet*, **i**, 1066.

Brief Communication

Contact Sensitivity to Sorbic acid in 'Unguentum Merck'

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A 31-year-old housewife had suffered from psoriasis for 13 years. It had been particularly troublesome on her hands and feet. An ointment composed of equal parts of clobetasol propionate 0.05% and 'Unguentum Merck' (E. Merck Ltd.) was prescribed for these areas. For about 10 months there was a good response to this treatment, but later it was changed to a different steroid ointment due to an apparent erythematous deterioration of the psoriasis of hands and feet as well as the appearance of steroid atrophy on both wrists. About 2 weeks later 'Unguentum Merck' alone was applied to the same areas and within 12 h there was considerable erythema, soreness and pruritis in them.

Patch tests with the ICDRG series were negative but gave an allergic type of reaction to 'Unguentum Merck' and to one of its components as follows:

		48 h	72 h
Cetostearyl alcohol	10% in pet	—	—
Glycerol monostearate	30% in pet	—	—
Polysorbate 40	as is	—	—
Propylene glycol	10% aq. sol	—	—
Saturated neutral oil	25% o.o.	—	—
Silicic acid	10% in pet	—	—
	1% in pet	—	—
Sorbic acid	5% in pet	++	++
'Unguentum Merck'		++	+

COMMENT

'Unguentum Merck' is widely used both as an emollient and as a diluent of steroid creams and ointments. Although its excellent physical properties are understood by prescribers, its chemical composition is often overlooked, hence the possibility of an allergic contact dermatitis due to sorbic acid or one of its other constituents is not considered. In 3 years of marketing Messrs Merck have been informed of only four apparent skin reactions to it. Two of these patients had an allergic reaction to sorbic acid (Cooper & Wood, 1977).

Sorbic acid 0.2% as contained in 'Unguentum Merck' is considered to be the antimicrobial agent of choice for topically applied vehicles and cosmetics containing fatty acids and polyoxyethylene esters (Fisher, 1973). However, the sensitizing index for sorbic acid is estimated to be 0.3% (Hjorth & Trolle-Lasen, 1962) and 0.6% (Klaschka & Biersdorff, 1965). Rudzki & Kleniewska (1970) noted 1.6% positive reactions to sorbic acid (3%) in a group of 436 eczematous patients. Fisher *et al.* (1971) found that out of 100 patients suspected of having an allergic contact dermatitis due to topically applied medications only one showed a positive patch test reaction to 5% sorbic acid.

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REFERENCES

- COOPER, A.J. & WOOD, D.A. (1977) From E. Merck Ltd., Personal communications.
- FISHER, A.A., PASCHER, F. & KANOF, N.B. (1971) Allergic contact dermatitis due to ingredients of vehicles. *Archives of Dermatology*, **104**, 286.
- FISHER, A.A. (1973) *Contact Dermatitis*, 2nd edn., p. 56. Lea & Febiger, Philadelphia.
- HJORTH, N. & TROLLE-LASEN, C. (1962) Skin reactions to preservatives in cream with special regard to paraben esters and sorbic acid. *American Perfumer*, **77**, 43.
- KLASCHKA, F. & BIERSDORFF, H. (1965) Allergic eczematous reaction for sorbic acid used as a preservative in external medicaments. *Münchener Medizinische Wochenschrift*, **107**, 185.
- RUDZKI, E. & KLENIEWSKA, D. (1970) The epidemiology of contact dermatitis in Poland. *British Journal of Dermatology*, **83**, 543.

Brief Communication

Juvenile plantar dermatosis in identical twins

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Mackie & Husain (1976) have used the term juvenile plantar dermatosis to describe a chronic, and at times painful, dermatitis affecting the weight-bearing areas of the feet. They carried out a comprehensive investigation of this disorder. Despite the clinical appearance suggestive of an allergic contact dermatitis, only about 1 in 9 children had positive patch tests to any substance in the European battery or to constituents of their own footwear. Nor could a fungal cause be substantiated. These workers were of the opinion that a likely aetiological factor is the increasing use of synthetic materials for children's socks. This condition appears to have become much more common in paediatric dermatology clinics in recent years. The cause of this dermatosis is not known.

Millard & Gould (1977) suggested that a number of factors are playing a role in the development of this dermatosis including an atopic diathesis and the infrequent changing of nylon or nylon/wool socks and canvas/rubber training shoes.

Hanifin (1978) has recently reported a highly potent contact allergen present in the adhesive, a component of the insole of athletic shoes.

Verbov (1978) examined 104 children with atopic eczema and noted the condition localized to the forefoot in twelve. He is of the opinion that juvenile plantar dermatosis is an expression of atopic eczema. However, it is likely that some patients with atopic eczema, a very common skin disorder, will also have juvenile plantar dermatosis.

CASE REPORTS

A girl of 10 attended with eczema of the feet present for 1 year. Her father suffered from asthma. At the time of interview the patient's identical twin sister, who attended the clinic, had not complained of any trouble with the feet and wore identical footwear. On close questioning, the only difference between the two girls, according to the mother, was that the patient was a 'bit of a tomboy' and liked to play football with the boys. Examination of the patient revealed classical juvenile plantar dermatosis while examination of the twin sister revealed a similar but less marked condition.

DISCUSSION

The cause of juvenile plantar dermatosis is still not settled and it is incautious to make generalizations from findings in isolated cases. However, the dermatosis has not been reported in identical twins and the findings suggest that both an inherited predisposition and an external factor, possibly trauma, are involved.

REFERENCES

- HANIFIN, J.M. (1978) Nike training shoe dermatitis. *Archives of Dermatology*, **114**, 289.
MACKIE, R.M. & HUSAIN, S.L. (1976) Juvenile plantar dermatosis: A new entity? *Clinical and Experimental Dermatology*, **1**, 253.
MILLARD, L.G. & GOULD, D.J. (1977) Juvenile plantar dermatosis. *Clinical and Experimental Dermatology*, **2**, 186.
VERBOV, J. (1978) Atopic eczema localized to forefoot. An unrecognized entity. *Practitioner*, **220**, 465.

Comment

CHROMIUM AND THE SKIN

Chromium has been of special concern to dermatologists since 1827, when William Cummin in Scotland first described chrome ulcers, but there has been a special interest since Hermanni in 1901 showed that contact dermatitis could be produced by chromium. It took a further 24 years until Parkhurst (1925) showed by patch testing that the dermatitis was due to a contact allergy. Subsequent investigation has mainly been concerned with discovering fresh sources of sensitization and with the discussion as to whether patients with dermatitis became sensitized to tri- or hexa-valent chromium. Toxic effects of chromium, however, are of much wider interest and have been incriminated in nasal ulceration, bronchitis, and carcinogenesis particularly of the lung.

Chromium has six valence states in special organic complexes but four of these are more or less unstable as ions; the two important ones are tri- and hexa-valent chromium. Schroeder *et al.* (1962) have given a very full review of the distribution of chromium in the environment. Chromium is distributed widely both in the earth and the sea and it ranks fourth among the 29 elements of biological importance in the earth's crust, being more abundant than cobalt, copper, zinc, molybdenum, lead, nickel and cadmium. In the earth's crust and seawater its average concentration is 200 ppm and 0.00004–0.00046 ppm respectively.

Minute quantities of chromium are also found in food, viz: dairy products 0.10 mean $\mu\text{g/g}$; meat and fish mean 0.11 $\mu\text{g/g}$, vegetables mean 0.03 $\mu\text{g/g}$, fruit mean 0.02 $\mu\text{g/g}$, grain mean 0.04 $\mu\text{g/g}$. The highest concentrations in food occur in thyme 10.00 $\mu\text{g/g}$, black pepper 3.3 $\mu\text{g/g}$ and cloves 1.50 $\mu\text{g/g}$. Animal diets contain higher amounts, e.g. dog food pellets 4.24 $\mu\text{g/g}$. Other vegetation unlikely to be eaten, such as leaves of trees, contain much higher quantities, for instance, wild cherry leaves 0.57 $\mu\text{g/g}$, pasture grass 1.30 $\mu\text{g/g}$. Cigarettes contain 0.39 $\mu\text{g/g}$, drinking water 0–0.112 ppm. Total human body content is about 6 mg of chromium; there is a higher concentration in infants and animals generally have very much higher concentrations. There are three reasons for the low concentration in the human body:

- (1) Most chromium in earth is in the insoluble chromite form.
- (2) Vegetation consumed by humans tends to be that with the lowest concentration.
- (3) Any hexavalent chromium which is absorbed is changed in the acid media of the stomach to the trivalent form which is not absorbed (Donaldson & Barreras, 1966).

In the United States, use of imported chromium is divided in the following way (U.S. National Research Council)—metallurgical industry 57%, mainly trivalent—stainless steel and alloy sheets; refractory materials 30%, mainly trivalent—furnace bricks—this use is declining as open hearths are being replaced; chemical industry 13%—tanning agents, pigments, catalysts, plating and wood preservatives. Chief world producers are USSR, South Africa, The Philippines and Rhodesia.

The function of chromium in the body is not clear but it does appear to be essential. Mertz (1969) has thoroughly reviewed the biological effects of chrome. It has been shown to enhance two metabolic processes, lipid and glucose synthesis. Curran (1954) showed increases of cholesterol and fatty acid synthesis from acetate by rat liver both *in vitro* and *in vivo* with chromium. Schwarz & Mertz (1959) showed that rats deficient in chromium had a lower tolerance to glucose and showed that *in vitro*, chromium with insulin markedly enhanced the incorporation of glucose into fat and less so of acetate. Low chromium states do exist as shown by cases of chromium responsive impairment of glucose

tolerance but it does not improve glucose metabolism to such a degree that hypoglycaemic medication had to be reduced (Mertz & Glinsmann, 1964; Glinsmann & Mertz, 1966).

TOXIC EFFECTS OF CHROME

- (1) Chronic bronchitis.
- (2) Carcinogenesis particularly in the lungs.
- (3) Ulceration of skin and nasal septum.
- (4) Dermatitis.

Chronic bronchitis

There has been some controversy about whether chromium can produce bronchitis and asthma. Brinton *et al.* (1952), investigating workers in the chromate producing industry, found no radiological evidence of disease and no decrease in vital capacity. However, Reggiani *et al.* in 1973, studying workers exposed to chromic acid, found 60% with subjective symptoms of chronic bronchitis though spirographic changes were detected in only a small number of cases. However, investigation of bronchospastic activity showed a significant increase in about 25% of subjects and CO₂ transfer was significantly decreased in over 60% of cases. Respiratory symptoms occur both in those employed in the production of chromate and bichromate from chromite ore (Krishna *et al.*, 1976) and in chromium platers where the asthma might occur as the result of allergy rather than a direct toxic effect on the respiratory mucosa (Royle, 1975).

Carcinogenesis

Evidence for carcinogenesis:

- (a) Medium solubility hexavalent chromium salts loaded on stainless steel pellets implanted in rat bronchioles produced squamous cell carcinoma (Levy & Venitt, 1975).
- (b) Levis & Buttigno (1977) showed that potassium dichromate interferes with DNA synthesis in hamster fibroblasts.
- (c) Epidemiological surveys both in the U.S. (Malche & Gregorious, 1948) and in Great Britain (Bidstrup & Case, 1956) confirmed an increased incidence of lung cancer in men employed in the production of chromate and bichromate from chromite ore. Recently, however, there has been evidence that this carcinogenic effect also occurs in the chromium platers. Waterhouse (1975) in a study of over 4000 workers exposed to the hazards of chromium plating found 49 deaths from lung cancer, which was significantly above ($P > 0.05$) the expected figure of 35. Royle (1975), in a retrospective study in chromium plating in the West Riding of Yorkshire, investigated 1238 chromium platers and 1282 controls. He found a statistically significant increase in neoplasms of all organs and of the lungs in particular. (All organs—platers 39, controls 21; lung—platers 18, controls 13).

Skin and nasal ulceration

The irritative and corrosive action on the skin and nasal mucosae of chromic acid has been well known since the 19th century and since 1919 in the United Kingdom there has been a statutory obligation to report cases. Though the reduction in the number of cases reported in the last 10 years has been impressive, the level of the cases is still around that found in the 1930's at about 100 a year. As opposed to dermatitis, skin ulcers, nasal ulcers and nasal perforations multiply as both the length of time and concentration increase (Royle, 1975). Royle (1975) in a retrospective study of 997 platers employed in the West Riding of Yorkshire found that 21.8% had skin ulcers, 12.8% nasal ulcers, and 4.7% nasal perforation. However, when hygienic conditions were poor this could rise to 34.1% skin ulcers and

61.0% nasal ulceration. Krishna *et al.* (1976) found a very high incidence (61.6%) of skin ulcers in recent employees working in chromium manufacture. Such a high incidence must indicate a very high turnover of staff in these high risk occupations.

It would appear that the present United Kingdom regulations are not sufficient to prevent ulceration. They consist of

- (1) Tests carried out every 14 days to ensure that environmental levels are not above the following:

Soluble chromic, chromous salts, as Cr—0.5 mg/m³ of air.

Chromates, dichromates, chromic acid—0.1 mg/m³ of air.

Chromium metal and insoluble salts—1.0 mg/m³ of air, excluding hexavalent chromium compounds.

- (2) Medical examinations once a month.

- (3) Regular inspection of hands and forearms.

(4) Various preventative measures, gloves, washing facilities and application of an ointment as follows to the uncovered part (soft paraffin 3 parts, lanolin 1 part). One wonders how many cases of chromium dermatitis are in fact lanolin dermatitis. With 100 cases of skin ulceration yearly and the increased incidence of carcinoma of the lung in platers, the current regulations in the United Kingdom do not appear adequate.

Contact dermatitis

Chromium is one of the commonest causes of contact dermatitis, producing 6.6% of positive patch tests in the analysis of 4825 cases from eight European centres, coming third behind wood tar and nickel (Fregert *et al.*, 1969). However, its main interest is probably because those who develop chromium dermatitis have a bleak outlook dermatologically and because few other substances will sensitize with such small concentrations even though it is not a potent sensitizer. Indeed, some suggest that it sensitizes better at lower concentrations (Polak *et al.*, 1973). However, the figures for dermatitis in chrome platers would not confirm this (Royle, 1975).

Incidence and trends. In the 1950's and 60's there was a substantial increase in the number of cases of chromium dermatitis. In some areas there was a 3 or 4-fold increase (Kurzawa *et al.*, 1971; Neubert, 1971). This is probably due to the increase in activity in the chromium industry, e.g. total usage of chrome in the United States was as follows: 1952—343,000 tons, 1956—566,000 tons, 1968—307,000 tons. Chromium ulcer notification in the United Kingdom was also at its height at that time. In the last few years there have been signs of a significant drop, e.g. fall in cases of cement dermatitis in Northern Ireland (Burrows & Corbett, 1977) and Sweden (Fregert, 1977).

Causes of chromium dermatitis. The following occupations are well known to have a chromium dermatitis risk: cement workers, chromium platers, dyers, printing industry, photographic and men working with anti-rust agents, wood impregnated with chromium. The following further occupations have been described in recent years as carrying a risk of dermatitis: defatting solvent (Ros & Bang-Pedersen, 1977), food laboratory (Bang-Pedersen, 1977), oil from galvanized sheet metal (Fregert & Gruvberger, 1973), postage stamps (Fregert, Gruvberger & Mitchell, 1975), coolant oils (Einarsson *et al.*, 1975), offset printing (Malten 1975), blackjack green beige tabletops (Fisher, 1976), milk testers (Rudsky & Czerwinska-Dihnz, 1977; Hureiz, Martin & Lefebvre, 1975; Rogers & Burrows, 1975), TV screens (Stevenson, 1975), shoes (Varelzides *et al.*, 1974), quick lime (Weiler & Russell, 1974), magnetic tapes (Krook *et al.*, 1977), detergents and bleaches (Garcia-Perez *et*

al., 1973) boiler linings (Rycroft & Calnan, 1977)—surprisingly only one case in spite of 30% used in refractory materials.

Significance of positive chromium test. Positive patch tests to chromium occur which do not seem to be relevant to the patient's condition and where there is no obvious contact with chromium. The European Contact Dermatitis Research Group considered that only 71% of their positive patch tests to chromium were significant. Probably, as more sources of contact with chromium are discovered, this percentage will increase. However, it will probably never reach 100%, particularly as a positive patch test occurs in about 5% of healthy cement workers (Amrein & Miecher, 1955; Hunziker & Musso, 1960; Burrows & Calnan, 1965). Walsh (1953) found three positive patch tests to 1% potassium dichromate among 60 patients in a tuberculosis sanatorium. Surveys of patients considered to have eczema and with no obvious contact with chromium have positive patch tests in percentages varying from 0 to 12% (Bett, 1958; Engelhardt & Mayer, 1949; Jaeger & Pelloni, 1950; Anderson, 1960; Hunziker & Musso, 1960; Stevenson, 1960).

TABLE 1

Occupation	No.	Positive to chromium only	Significant
Student/school	4	—	—
Nurse	4	1	—
Housewife	4	—	—
Fitter/welder	4	1	2
Cook/chef	3	1	—
Director of rubber firm	1	—	—
Manager of cement firm	1	—	1
Scaffolder	1	—	1
Bookkeeper	1	—	—
Joiner	1	1	1
Teacher (caused by dye in shirt)	1	1	1
Assembly worker	1	—	1
Plasterer	1	1	1
Terazzo floor layer	1	1	1

Fresh sources of contact with chromium are still being discovered and it may be that many of these cases of 'eczema' are in fact cases of contact dermatitis due to a non-detected contact with chromium, particularly as chromium dermatitis can produce patterns which resemble constitutional eczema (Burrows & Calnan, 1965). In 1977 in the Royal Victoria Hospital, 28 (5.3%) positive patch tests to chromium were found at 48 h in 529 patients patch tested to the European Battery; nickel (80), wood tars (59), thiuram mix (31). Other centres carrying out patch tests with the European Battery have found a similar percentage—(Fregert *et al.*, 1969; Husain, 1977). It would therefore appear that about the same number of positives to chromium are observed whether one tests groups of patients with normal skin or patients with skin diseases suspected of having contact dermatitis from whatever cause. This suggests that a large number of positive tests obtained in routine battery testing must be non-significant. In only nine (33%) of our cases was the rash consistent with contact dermatitis and there was an obvious contact with chromium (see Table 1). Seven were positive to chromium alone and

of these, four (57%) were considered significant. A high percentage of chromium positives has been found in 'housewife's dermatitis' in some countries (Spain and Israel, and contact with chromium in detergents and bleaches was considered significant (Feuerman, 1969; Garcia-Perez *et al.*, 1973). It may be, therefore, that nurses and housewives ought to be considered to have 'significant' chromium contact. None showed light sensitivity (Wahlberg & Wennersten, 1977).

Multiple sensitivities. In the Royal Victoria Hospital figures for 1977, only 7 of 28 patients with a positive patch test to chromium were positive to chromium alone. The other associated allergens were as follows:

Nickel	13
Cobalt	9
Rubber	6
Colophony	2
Neomycin	1
Balsam of Peru	1
Epoxy resin	1
'QED' (detergent)	1

There are three explanations for these multiple sensitivities:

(1) That the patient is indeed truly allergic to several substances. This is clearly important in some cases. It is supported by experimental work and by the fact that the associated positives are grouped, e.g. other metals in chromium sensitivity, medicaments and local applications in various ulcers, and rubber chemicals. This suggests that simultaneous contact is an important factor.

(2) Angry back syndrome. Mitchell (1975, 1977) has shown clearly that a positive reaction under one patch in the battery will stimulate reactions under the others but when these are tested separately no reaction is produced.

(3) Pautrizel *et al.* (1962) have suggested that persons allergic to chromium do not react with the chromium but to the altered protein to which it is joined as a hapten. Cohen (1976) postulates that other metals will alter proteins to which they are attached in a similar way and that the body recognizes the altered protein to which the chromium is attached rather than the full complex.

Hexavalent vs trivalent. It is very difficult to sensitize with trivalent chromium; it takes somewhere about twenty times the amount for epicutaneous sensitization and fifty times for intracutaneous sensitization (Neer, 1965). Yet all immunological methods of detection of sensitization apart from patch testing and macrophage migration would indicate that trivalent chromium is the metal involved (Fregert & Rorsman, 1964; Cohen, 1966; Mali *et al.*, 1966; Polak *et al.*, 1973). The current concept is that trivalent chromium readily complexes with proteins in the skin and so does not penetrate easily (Anderson, 1960). Hexavalent chromium does not form complexes with organic substances but readily penetrates the epidermis to the dermis (Mali *et al.*, 1964; Czernilewski *et al.*, 1965). Indeed trivalent chromium penetrates 10,000 times less (Spruit & Neer, 1966). Somewhere it is changed into trivalent chromium which readily complexes with the proteins there. One gram of skin can reduce 1.06 mg of hexavalent dichromate to trivalent chromium (Samitz & Katz, 1963, 1964). Polak *et al.* (1973) have thoroughly reviewed the literature on chromium sensitization, whether hexavalent or trivalent. It may be that this concept will have to be reassessed now that the Langerhans

cell is considered an antigen processor and that trivalent chromium applied to the surface of the skin readily concentrates around it (Shelley & Juhlin, 1977).

Prognosis. It is well recognized and documented (Beit & Turk, 1976) that prognosis in chromium dermatitis is bad and that the skin of a large percentage of people who get chromium dermatitis never completely or permanently clears up. Fregert (1975) showed that, of patients with industrial dermatitis, only 7% of female patients with chromium dermatitis completely cleared up and only 10% of men healed. In a series of patients who had made claims against their employers and who were followed up 10–13 years later a total of about 20% of patients with dermatitis from various causes were clear whereas in cement dermatitis cases, who were mostly chrome sensitive, only 6% were completely cleared (Burrows, 1972).

Prevention. (1) Definition and elimination of chromium from the environment or industrial processes, e.g. use another preservative instead of pot. bichromate in milk testers; avoid using chromium containing compounds as anti-rust agents. The diphenyl carbazide spot test may be useful.

(2) Change chromium to its less active sensitizing form (trivalent), e.g. addition of ferrous sulphate to cement (Fregert & Gruvberger, 1973).

(3) Improve preventative facilities, e.g. washing facilities on building sites, protective clothing, ventilation to reduce chromium in the atmosphere in plating factories.

(4) Mechanization where possible, e.g. this has eliminated chromium dermatitis in milk testers.

(5) Reduce amount of chromium in diet. It has been shown that small quantities of potassium dichromate taken orally will produce an exacerbation of chromium dermatitis (Fregert, 1965; Kaaber & Veien, 1977). This is surprising in view of the findings of Donaldson & Barreras (1966) who showed that most chromium is changed in the acid media of the stomach to trivalent chromium and not absorbed. Perhaps people with bad chromium dermatitis are achlorhydric. Fregert (1978) in an unpublished study of 20 cases of chromium dermatitis found that none was achlorhydric. However, in view of the very small quantity of chromium in the diet anyway, apart from avoiding cloves, thyme and pepper, it would be difficult to reduce intake much further. Whether chromium can be dissolved out of stainless steel in cooking seems still to be unresolved (Fregert, 1974; Christie *et al.*, 1976).

(6) Desensitization. Guinea-pigs allergic to chromium can be desensitized only in the following way: one intravenous injection of hexavalent chromium, 20 mg/kg, and then a skin test within 24 h with the same substance (Polak *et al.*, 1973). However, the intravenous dose is about LD₅₀ for the animals and so this approach is unlikely to be helpful in humans. Immunological tolerance to chromium in animals is possible by injection of 10 mg/kg potassium dichromate, 28 and 14 days before exposure to the sensitizer. It is difficult to conceive of a situation which would justify this pre-treatment of workers exposed to chrome.

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REFERENCES

- AMREIN, H.P. & MIESCHER, G. (1955) Zür Ätiologie des Zementekzems; Reihenuntersuchungen über die Häufigkeit einer Chromatsensibilisierung im Maurergewerbe. *Zeitschrift für Unfallmedizin und Berufskrankheiten*, **48**, 140.
- ANDERSON, F.E. (1960) Cement and oil dermatitis. The part played by chrome sensitivity. *British Journal of Dermatology*, **72**, 108.

- BANG-PEDERSEN, N.B. (1977) Chromate in the food laboratory. *Contact Dermatitis*, **3**, 105.
- BEIT, R. & TURK, R.B. (1976) The medical and social fate of the dichromate allergic patient. *British Journal of Dermatology*, **94**, 349.
- BETT, D.C.G. (1958) The potassium dichromate patch test. *Transactions of St John's Hospital Dermatological Society*, **40**, 40.
- BIDSTRUP, P.L. & CASE, R.A. (1956) Carcinoma of the lung in workmen in bichromates-producing industry in Great Britain. *British Journal of Industrial Medicine*, **13**, 260.
- BRINTON, H.P., FRASIER, E.S. & KOVEN, A.L. (1952) Morbidity and mortality experience among chromate workers. *Public Health Report*, **67**, 835.
- BURROWS, D. (1972) Prognosis in industrial dermatitis. *British Journal of Dermatology*, **87**, 145.
- BURROWS, D. & CALNAN, C.D. (1965) Cement dermatitis. II. Clinical aspects. *Transactions of St John's Hospital Dermatological Society*, **51**, 27.
- BURROWS, D. & CORBETT, J.R. (1977) Industrial dermatitis in Northern Ireland. *Contact Dermatitis*, **3**, 148.
- CHRISTIE, O.H.J., DINH-NGUYEN, N. & VINCENT, J. (1976) Spark source mass spectrographic study of metal allergic substances on the skin. *Journal of Investigative Dermatology*, **67**, 587.
- COHEN, H.A. (1966) Carrier specificity of tuberculin-type reaction to trivalent chromium. *Archives of Dermatology*, **93**, 34.
- COHEN, H.A. (1976) The role of carrier in sensitivity to chromium and cobalt. *Archives of Dermatology*, **112**, 37.
- CUMMIN, W. (1827) Remarks on the medicinal properties of Madar and on the effects of bichromate potassium on the human body. *Edinburgh Medical and Surgical Journal*, **28**, 295.
- CURRAN, G.L. (1954) Effect of certain transition group elements on hepatic synthesis of cholesterol in the rat. *Journal of Biological Chemistry*, **210**, 765.
- CZERNILEWSKI, A., BRYKOLSKI, D. & DEPCZYK, D. (1965) Experimental investigations on penetration of radioactive chromium (^{51}Cr) through the skin. *Dermatologica*, **131**, 334.
- DONALDSON, R.M. & BARRERAS, R.F. (1966) Intestinal absorption of trace elements of chromium. *Journal of Laboratory and Clinical Medicine*, **68**, 484.
- EINARSSON, O., KYLIN, B., LINDSTEDT, G. & WAHLBERG, J. E. (1975) Chromium, cobalt and nickel in used cutting fluids. *Contact Dermatitis*, **1**, 182.
- ENGELHARDT, W.E. & MAYER, R.L. (1931) Über Chromekzem im graphischen Gewerbe. *Archiv für Gewerbepathologie und Gewerbehygiene*, **2**, 140.
- FEUERMAN, E.J. (1969) Housewives' eczema and the role of chromates. *Acta dermato-venereologica*, **49**, 288.
- FISHER, A. (1976) Blackjack disease and other chromate puzzles. *Cutis*, **18**, 21.
- FREGERT, S. (1965) Separatum—Congress of the Hungarian Dermatological Society.
- FREGERT, S. (1974) *Manual of Contact Dermatitis*, p. 21. Munksgaard, Copenhagen.
- FREGERT, S. (1975) Occupational dermatitis in a 10-year material. *Contact Dermatitis*, **1**, 96.
- FREGERT, S. (1977) Personal communication.
- FREGERT, S. (1978) Unpublished data.
- FREGERT, S. & RORSMAN, H. (1964) Allergy to trivalent chromium. *Archives of Dermatology*, **90**, 4.
- FREGERT, S., HJORTH, N., MAGNUSSON, B. *et al.* (1969) Epidemiology of contact dermatitis. *Transactions of St John's Hospital Dermatological Society*, **55**, 17.
- FREGERT, S. & GRUVBERGER, B. (1973) Factors decreasing the content of water-soluble chromate in cement. *Acta dermato-venereologica*, **53**, 267.
- FREGERT, S., GRUVBERGER, B. & MITCHELL, J.C. (1975) Chromate in postage stamps. *Contact Dermatitis*, **1**, 328.
- GARCIA-PEREZ, A., MARTIN-PASCUAL, A. & SANCHEZ-MISERO, A. (1973) Chrome content in bleaches and detergents: its relationship to hard dermatitis in women. *Acta dermato-venereologica*, **53**, 353.
- GLINSMANN, W.H. & MERTZ, W.H. (1966) Effect of trivalent chromium on glucose tolerance. *Metabolism*, **15**, 105.
- HERMANNI, F. (1901) Die Erkrankungen der in Chromatfabriken beschäftigten Arbeiter. *Munchener medizinische Wochenschrift*, **48**, 536.
- HUNZIKER, N. & MUSSO, E. (1960) A propos de l'eczéma au ciment. *Dermatologica*, **121**, 204.
- HURIEZ, C.L., MAETIN, P. & LEFEBVRE, M. (1975) Sensitivity to dichromate in a milk analysis laboratory. *Contact Dermatitis*, **1**, 247.
- HUSAIN, S.L. (1977) Contact dermatitis in the West of Scotland. *Contact Dermatitis*, **3**, 327.
- JAEGER, H. & PELLONI, E. (1950) Tests épicutanés aux bichromates, positifs dans l'eczéma au ciment. *Dermatologica*, **100**, 207.
- KAABER, K. & VEIEN, N.K. (1977) The significance of chromate ingestion in patients allergic to chromate. *Acta dermato-venereologica*, **57**, 321.

- KRISHNA, G., MATHUR, J.S. & GUPTA, R.K. (1976) Health hazard amongst chrome industry workers with special reference to nasal septum perforation. *Indian Journal of Medical Research*, **64**, 866.
- KROOK, G., FREGERT, S. & GRUVBERGER, B. (1977) Chromate and cobalt eczema due to magnetic tapes. *Contact Dermatitis*, **3**, 60.
- KURZAWA B., QUAAS, M. & HOLLAX, R. (1971) Zur Epidemiologie der arbeitsbedingten Hautschädigungen durch Chromate unter besonderer Berücksichtigung der Rehabilitation und prophylaxe. *Deutsche Gesundheitswesen*, **26**, 81.
- LEWIS, A.G. & BUTTIGOL, M. (1977) The effects of potassium dichromate on DNA synthesis in Hamster fibroblasts. *British Journal of Cancer*, **35**, 496.
- LEVY, L.S. & VENITT, S. (1975) Carcinogenic and mutagenic activity of chromium containing materials. *British Journal of Cancer*, **32**, 254.
- MACHLE, W. & GREGORIUS, F. (1948) Cancer of respiratory system in United States chromate producing industry. *Public Health Report*, **63**, 1114.
- MALI, J.W.H., MALTEN, K. & VAN NEER, F.C.J. (1966) Allergy to chromium. *Archives of Dermatology*, **93**, 41.
- MALTEN, K.E. (1975) Cobalt and chromium in offset printing. *Contact Dermatitis*, **1**, 120.
- MERTZ, W. (1969) Chromium occurrence and function in biological systems. *Physiological Reviews*, **49**, 163.
- MERTZ, W. & GLINSMANN, W.H. (1964) The effect of trivalent chromium on glucose utilization in diabetes mellitus. In: *5th Congress of the International Diabetes Federation*, p. 137.
- MITCHELL, J.C. (1975) The angry back syndrome: eczema creates eczema. *Contact Dermatitis*, **1**, 193.
- MITCHELL, J.C. (1977) Multiple concomitant positive patch test reactions. *Contact Dermatitis*, **3**, 315.
- National Research Council Committee on Biological Effects of Atmospheric Pollutents. Medical and biological effects of environmental pollutants, chromium. *National Academy of Sciences*, 1974.
- NEER, F.C.J. VAN (1965) Reacties op intracutane injecties van drie en zeswaardige chroomverbindingen bij gesensibiliseerde mensen, vakkens en caviae. *Nederlandsch tijdschrift voor geneeskunde*, **189**, 1684.
- NEUBERT, H. (1971) Hauterkrankungen in der gewerblichen Wirtschaft. Statistischer Überblick. *Berufsdermatosen*, **19**, 28.
- PARKHURST, H.J. (1925) Dermatitis industrialis in a blueprint worker due to chromium compounds. *Archives of Dermatology and Syphilology*, **12**, 253.
- PAUTRIZEL, R., RIVASSEAU, J. & RIVASSEAU-COUTANT, A. (1962) Le traitement des allergies cutanées de contact par épidermodésensibilisation. *Médecine et hygiène*, **533**, 47.
- POLAK, L., TURK, J.L. & FREY, J.R. (1973) Studies on contact hypersensitivity to chromium compounds. *Progress in Allergy*, **17**, 145.
- REGGIANI, A., LOTTI, M., DE ROSA, E. & SAIA, B. (1973) Respiratory functional changes in subjects exposed to chromium. I. Spirographic changes. *Lavoro Umano*, **25**, 23.
- ROGERS, S. & BURROWS, D. (1975) Contact dermatitis to chrome in milk testers. *Contact Dermatitis*, **1**, 387.
- ROS, A.M. & BANG-PEDERSEN, N.B. (1977) Chromate in a defatting solvent. *Contact Dermatitis*, **3**, 105.
- ROYLE, H. (1975) Toxicity of chromic acid in the chromium plating industry. *Environmental Research*, **10**, 141.
- RUDSKY, E. & CZERWINSKA-DIHNZ, I. (1977) Sensitivity to dichromate milk testers: 6 cases of chromate allergy in milk testers. *Contact Dermatitis*, **3**, 107.
- RYCROFT, R.J.R. & CALNAN, C.D. (1977) Chromate dermatitis from a boiler lining. *Contact Dermatitis*, **3**, 198.
- SAMITZ, M.H. & KATZ, S.A. (1963) Preliminary studies on the reduction and binding of chromium with skin. *Archives of Dermatology*, **88**, 816.
- SAMITZ, M.H. & KATZ, S. (1964) A study of the chromical reactions between chromium and skin. *Journal of Investigative Dermatology*, **43**, 35.
- SCHROEDER, H.A., BALASSA, J.C. & TIPTON, I.H. (1962) Abnormal trace metals in man—chromium. *Journal of Chronic Diseases*, **15**, 941.
- SCHWARZ, K. & MERTZ, W. (1959) Chromium (III) and the glucose tolerance factor. *Archives of Biochemistry and Biophysics*, **85**, 292.
- SHELLEY, W.B. & JUHLIN, L. (1977) Selective uptake of contact allergens by the Langerhans cell. *Archives of Dermatology*, **113**, 187.
- STEVENSON, C. (1960) Personal communication.
- STEVENSON, C.J. (1975) Fluorescence as a clue to contamination in TV workers. *Contact Dermatitis*, **1**, 242.
- SPRUIT, D. & NEER, F.C.J. (1966) Penetration rate of Cr (III) and Cr (VI). *Dermatologica*, **132**, 179.
- VARELZIDES, A., KATSAMBAS, A., GEORGALA, S. *et al.* (1974) Shoe dermatitis in Greece. *Dermatologica*, **149**, 236.
- WAHLBERG, J.E. & WENNERSTEN, G. (1977) Light sensitivity and chromium dermatitis. *British Journal of Dermatology*, **97**, 411.

- WALSH, E.N. (1953) Chromate hazards in industry. *Journal of The American Medical Association*, **153**, 1305.
- WATERHOUSE, J.A.H. (1975) Cancer among chromium platers. *British Journal of Cancer*, **32**, 262.
- WEILER, K.-J. VON & RÜSSEL, H.A. (1974) Das Chromekzem durch Branntkalk. *Berufsdermatosen*, **22**, 116.

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Co-trimoxazole resistant *Mycobacterium marinum*

SIR, There have been a number of reports (Barrow & Hewitt, 1971; Kelly, 1976; Woolfson, Saunders & Meade, 1976; Black & Eykyn, 1977) on the value of Co-trimoxazole in the therapy of skin disease due to *Mycobacterium marinum*. However, a recent culture from a patient attending this hospital has proved resistant to this antibiotic. The patient, a 30-year-old woman, had lesions of the right wrist and elbow which had been present for two months at the first examination; in all there were four lesions illustrating the sporotrichoid spread associated with fish tank granuloma. The patient admitted that she kept tropical fish and that some had recently died.

Pus obtained from the lesion of the wrist was found to contain an acid fast bacillus growing at 30°C and resembling *M. marinum*. Confirmation of identity and antibiotic sensitivity tests were carried out by the Public Health Laboratory Service, Dulwich (Regional Centre for Tuberculosis Bacteriology) who reported *M. marinum* highly resistant to Streptomycin, INAH and PAS, resistant to Amikacin, Pyrazinamide and Septrin but sensitive to Ethambutol, Rifampicin and Erythromycin.

There was no attempt at antibiotic therapy prior to the patient attending this hospital. Subsequent to the PHLS report the patient received Erythromycin (1 g daily) and the lesions are resolving.

It is not possible, on the basis of this single report, to do other than warn those in charge of patients that Co-trimoxazole may not always be the antibiotic of choice. Recent reports in the Archives of Dermatology have suggested that Tetracycline may be efficacious, though this drug is rarely used in sensitivity tests conducted at mycobacterial reference centres. Where microbiology is attempted it is valuable to bear in mind the observations of Black & Eykyn (1977) that culture is much easier from dead or diseased fish than from the patient's lesion.

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REFERENCES

- BARROW, G.I. & HEWITT, M. (1971) Skin infection with *Mycobacterium marinum* from a tropical fish tank. *British Medical Journal*, **ii**, 505.
BLACK, M.M. & EYKYN, S.J. (1977) The successful treatment of tropical fish tank granuloma (*Mycobacterium marinum*) with Co-trimoxazole. *British Journal of Dermatology*, **97**, 689.
KELLY, R. (1976) *Mycobacterium marinum* infection from a tropical fish tank. Treatment with trimethoprim and sulphamethoxazole. *Medical Journal of Australia*, **ii**, 681.
WOOLFSON, H., SAUNDERS, K.E. & MEADE, G. (1976) Fish tank granuloma. *British Journal of Clinical Practice*, **30**, 122.

Book Reviews

Abrégé de Dermatologie et de Vénérologie. C.I.HURIEZ, F.DESMONS and H.BERGOEND. (1978) Paris: Masson. Pp. 476, 111 figs. 53 col. illus. Price 77 francs.

The senior author of this abridged text of dermatology has been one of the leading actors in the amphitheatre of French Dermatology since 1929. A visitor to Lille watching him at work in his home surroundings would be as conscious of his greatness as when seeing him take the stage at an International Congress usually in full and poetic voice. Great poets are not usually authors of the most concise or precise text books and the best sections of this text book are chapters such as that on the skin manifestations of diabetes mellitus which are more comprehensive than is usual in a manual of this size. There are several of those enormously detailed figures or diagrams which Professor Huriez has always enjoyed issuing to his audience. Like William Morris wallpapers they may become fashionable again in the near future.

Ninety-nine percent of the text expresses a view of dermatology which will be wholly acceptable to the British reader and the emphasis on general medicine is as characteristic of the author as it is of an audience in the U.K. The reviewer must admit that he reads this kind of text with a bias towards noting what is different to his own practice and so it is to therapy that one turns with interest. In some sections such as that on psoriasis the advice is 'wordy' but not a practical guide. How exactly does one use dithranol? P.U.V.A. is new. Since it is described to the new reader as 'plus rapides et plus constants', it needs more explanation on how to use it and how it is obtained.

What does the British reader make of hydrotherapy, massage and vitamins for young girls. What about arsenicals, tetracycline, Vitamin D, giving up coffee or receiving radiotherapy to the spine for those who have lichen planus.

One has to be a dermatologist to recognize 50% of the fuzzy illustrations. For the student who does not sit at the feet of Professor Huriez, the subject will remain a blur.

T.J.RYAN

Heme and Hemoproteins (Handbook of Experimental Pharmacology Vol. 44). Edited by FRANCESCO DE MATTEIS and W.NORMAN ALDRIDGE (1978) Berlin: Springer Verlag. Pp. 449, 60 figs. Price US \$95.00.

Contributions from many leading authorities in haem biochemistry and pharmacology have been combined to produce an informative though concise volume. Direct interest to the clinical dermatologist will be limited as the book concentrates almost exclusively on biochemical pathways in haem metabolism and the interaction of toxins and pharmacological agents thereon.

The editors have constructed the book in a logical fashion. The opening chapter usefully summarizes haem biosynthesis and degradation including an outline of the role of the individual enzymes involved and the control of synthesis in red cells and liver. Induction and inhibition of hepatic haemoprotein synthesis are subsequently discussed and thereafter the individual effects of foreign chemicals, hormonal influences and nutrition are described in a series of detailed chapters.

Perhaps of most relevance to skin disease is the chapter by Douglas Maxwell and Urs Meyer on pharmacogenetics in the field of haem metabolism. Some attempt is made to correlate the clinical features of the hepatic porphyrias with the biochemical facts. The precision with which the enzymic lesions have been defined is unfortunately not mirrored by accurate knowledge of the pathogenesis of the clinical manifestations.

It is a pity that the erythropoietic porphyrias have not been covered in greater detail and it is surprising for instance that no prominence is given to the role of deficient ferrochelatase in erythropoietic protoporphyria.

Despite occasional deficiencies from a dermatological view point, this edition is a useful reference book and certainly a worthwhile acquisition for those interested in porphyria.

D.M.MACDONALD

Dermatologie und Venerologie. Eine Propädeutik und Systematik. A. GREITHER, Third, improved and expanded edition (1978). Berlin: Springer. Pp. xviii + 212, 82 illustrations.

My favourable review of the first edition (*British Journal of Dermatology*, **82**, 652, 1973) praised this compact, clear and well arranged text with numerous schematic drawings but without a single clinical photograph.

This third corrected and expanded edition includes a new chapter on hair in health and disease and a few pages on andrology. Clearly, testicles and their disorders, male infertility and its understanding, command enough interest in Germany to merit a separate chapter.

I welcome the improvements and innovations, but I still think that there is no substitute for clinical photographs in a dermatological textbook and I miss that little extra spark of inspiration which would change this good little book into an excellent one.

I. SARKANY

Year Book of Dermatology 1977. Edited by FREDERICK D. MALKINSON and ROGER W. PEARSON (1978). Pp. 412, B/W illus. Price £18.75. New York: Year Book Medical Publishers.

The Year Book of Dermatology is one of the most important publications in Dermatology. It serves two main functions: firstly, it provides an easy method of being made aware of articles that one has missed, and of reminding one of articles that one has read but forgotten! Secondly, it provides an excellent source of reference presented in a most attractive and useful way. The editors' comments after many of the abstracts are particularly helpful, and are usually pertinent.

As in previous years, the book opens with a major review article which in 1977 is entitled 'Advances in the diagnosis and treatment of blistering diseases: a selective review', by Pearson. This is a most interesting and useful review but by the nature of the publication it is one to two years out of date, and there have been some important publications on the bullous diseases, especially on their immunopathology, in the last 2 years.

The book is presented in a clear and concise way and is a pleasure to read. Drs Malkinson and Pearson are to be congratulated on their maintenance of such a high standard. All dermatologists should have access to a copy and should preferably own one. However, the price is rising steeply: in 1975—£11.30; in 1976—£14.30 and in 1977—£18.75. It is likely that many dermatologists will not buy a copy this year, but without doubt they will be the losers.

A. P. WARIN

Clothing Comfort. Interaction of Thermal, Ventilation, Construction and Assessment Factors. Edited by N. R. S. HOLLIES and R. F. GOLDMAN (1978). Published by Ann Arbor Science, Pub. Inc. Michigan and distributed by John Wiley & Sons Ltd., Chichester. Pp. 189. Price £15.45.

This account of the Fibre Society symposium on the comfort of clothing comes from Army research establishments, Physiology and Textile university departments and US industry. One learns that by the end of the 16th century armour was so heavy that some troops refused to wear it. Nowadays high velocity bullet proof vests with ceramic plates may weigh 10 kilograms.

Most of the book is of more interest to the textile industry than to dermatologists, dealing as it does with the insulating properties of clothing in arctic and tropical conditions, the extensibility of fibres and the effect of ventilation and windspeed. Ingenious heated life-sized copper manikins have been devised which monitor surface temperature. Synthetic fibres which are being increasingly used for fabrics are mostly hydrophobic. There is an interesting account of the various methods used for imparting hydrophilic finishes to synthetic fibres.

K. GRICE

News and Notices

Occupational Dermatology Symposium 26-28 March 1979. University of California Hospital, San Francisco

This course will succinctly summarize principles and practices of occupational dermatology, stressing the latest relevant advances. The faculty of more than 50 are international in scope. Presentations are aimed at physicians, nurses, and allied health scientists involved in the prevention and treatment of occupational skin disease. Workshops and field trips are on an optional basis. The course is jointly sponsored by the I.C.D.R.A., N.A.C.D.A., N.I.O.S.H., and the University of California. For information, write or call, Extended Programs in Medical Education, University of California Hospital, San Francisco, California, 94143, U.S.A. Telephone (415) 666-4251.

International Symposium on Percutaneous Absorption of Steroids

The symposium will be held on 5-6 April 1979 in Paris, France. The main topics will be: (1) Percutaneous absorption of steroids including theoretical aspects, reservoir effect of skin, bioavailability and effects of different vehicles on percutaneous absorption. (2) Metabolism of steroids in skin. (3) Applications of percutaneous absorption of steroids in dermatology including hormonal control of sebaceous gland and hair growth; topical use of oestrogens, androgens, antiandrogens and corticosteroids. (4) Applications of percutaneous absorption of steroids in endocrinology.

International Organizing Committee: P. Mauvais-Jarvis (France), M.K. Polano (the Netherlands), C.F.H. Vickers (England), J. Wepierre (France).

The symposium will include invited lectures and a limited number of free communications. The official languages of the symposium will be English and French.

Information about the symposium and application forms can be obtained from: P. Mauvais-Jarvis, Hôpital Necker, Service d'Endocrinologie et de Gynécologie Médicale-149, rue de Sèvres 75730, Paris Cedex 15, France.

Annual Hair Transplant Symposium

The Annual Hair Transplant Symposium will be held in Hot Springs, Arkansas, U.S.A. on 25-27 January 1979. International Multidiscipline faculty composed of both dermatologists, otolaryngologists and plastic surgeons. Registration fee: \$720.

Inquiries can be made to: D.B. Stough, III, M.D., Program Chairman, Stough Dermatology and Cutaneous Surgery Clinic, P.A., Doctors Park, Hot Springs, Ark. 71901, U.S.A.

University of California Hospital, San Francisco

The Seventh Annual Consultant's Course will be held at the University of California Medical Center, San Francisco on Tuesday 6 March to Friday 9 March. On 10 March the course ends with the semi-annual meeting of the San Francisco Dermatologic Society at the medical center. The faculty largely consists of members of the Department of Dermatology. Major guest lectureships include the Frank Allende and Ingram lectureships.

For information, please write or call Extended Programs in Medical Education, University of California, San Francisco, California 94143 (415-666-4251), U.S.A.

Corrigendum

Leitfaden der Medizinischen Mykologie, 2nd Edn. by Herbert Koch (1977). In the book review which appeared in August 1978 (Vol. 99, p. 225) the publisher should have been given as Gustav Fischer Verlag, Jena.

International Coding Index for Dermatology

Compatible with the 9th Revision of the
WHO International Classification of Disease

Compiled by
Suzanne Alexander and Alan B. Shrank

This new index is designed to permit the accurate and efficient retrieval of information on dermatological diagnoses, and has a much wider scope than the index issued by the World Health Organization. The coding system is based upon the International Classification of Disease, which is understood and accepted universally, and it is entirely compatible with the Ninth Revision due to be inaugurated in 1979. It is compact yet inclusive of many new disorders, and will prove its value in epidemiological studies, in medical research and in the planning of health services. The coding system is becoming widely adopted throughout the world and dermatologists will therefore find this index to be increasingly useful in their practice.

1978. 112 pages. Paper. £2.00

Published for the
British Association of Dermatologists by
Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

Investigative Techniques in Dermatology

Edited by R. Marks M.B. B.Sc. F.R.C.P. Autumn 1978. 240 pages, 70 illustrations. About £10.00

This book provides a practical link between the purely theoretical works on the skin and its diseases and laboratory manuals. It particularly emphasises the advantages and disadvantages of commonly used techniques and how they can most effectively be employed.

Contents Looking at skin lipids; Technique of patch tests; Immune complexes; The investigation of fibrinolytic activity; Methods for microbiological investigation for acne; The investigation of photodermatoses; Identification of mediators of inflammation; Genetic techniques in dermatology; Transmission electron microscopy; In vitro tests of cell mediated immunity; Epidermal cell kinetics; The application of scanning electron microscopy to dermatological problems; Clinical trials in dermatology; Techniques for the examination of connective tissue.

Textbook of Dermatology

Edited by A. Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and F.J.G. Ebling D.Sc. Ph.D. Third Edition, Autumn 1978. 2094 pages, 1100 illustrations. Two volumes, about £80.00

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author to whom all correspondence should be sent should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to request minor corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μ g	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	131I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs.' and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

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British Journal of Dermatology, Volume 99, Number 5, November 1978

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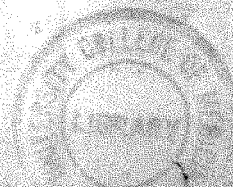
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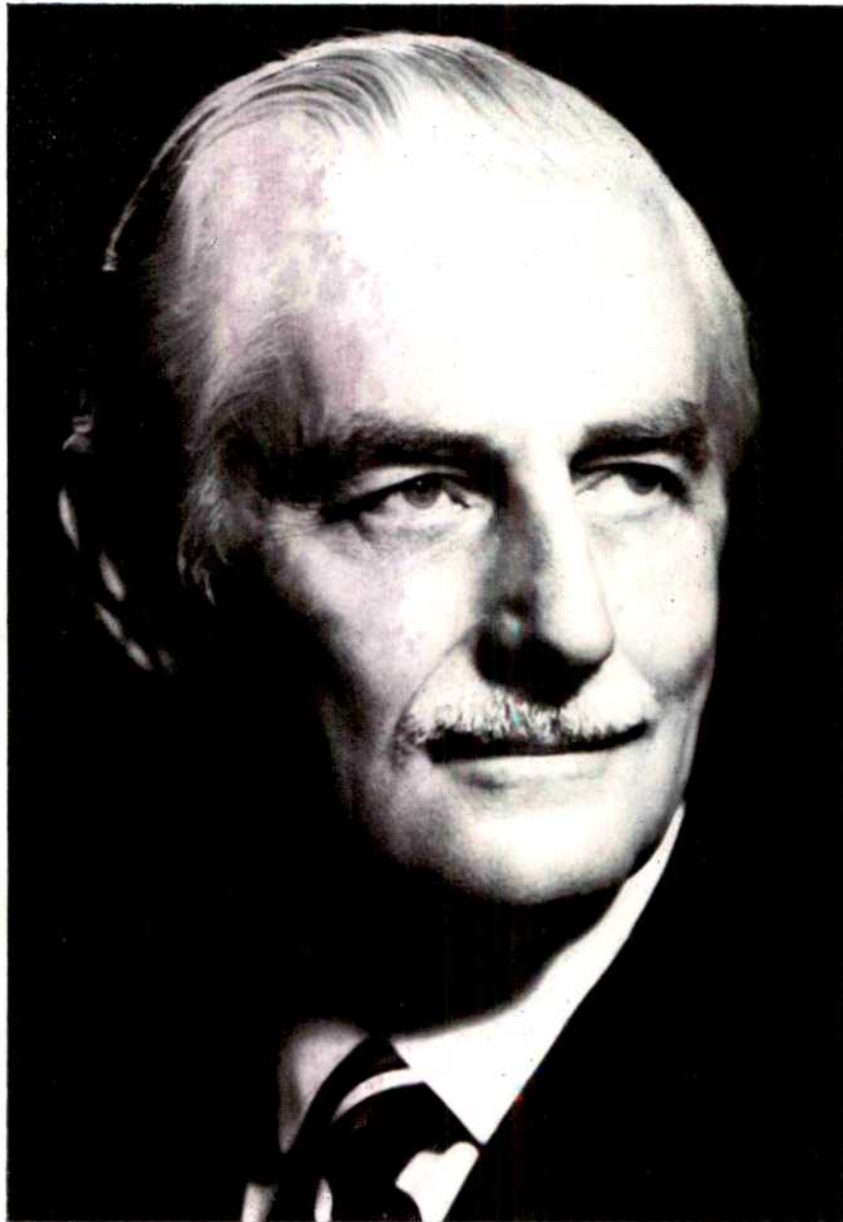
Foreword

As in previous years this supplement contains summaries of the papers, titles of the poster displays and details of the cases to be presented at the fifty-eighth annual meeting of the British Association of Dermatologists.

Those attending the meeting are asked to bring this Supplement with them.

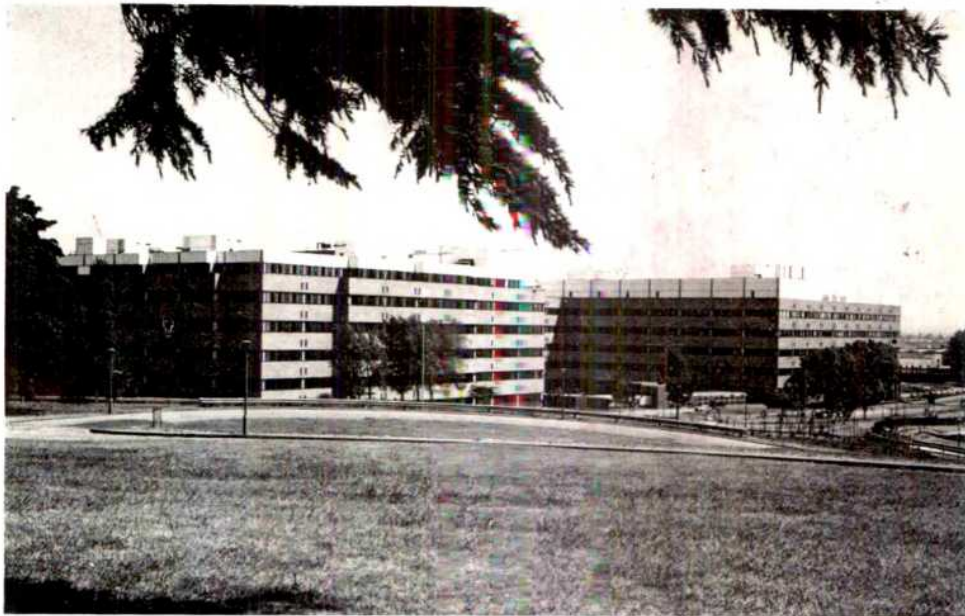
Once again we are extremely grateful to Glaxo Laboratories Limited for their financial support.

R.H. CHAMPION



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PRESIDENT OF THE BRITISH ASSOCIATION OF DERMATOLOGISTS, 1977-8



QUEEN'S MEDICAL CENTRE, NOTTINGHAM
Architects: Building Design Partnership

British Association of Dermatologists Fifty-Eighth Annual Meeting, 1978 Nottingham

PRESIDENT: DR PATRICK D. C. KINMONT,
M.B.E., T.D.

PROGRAMME OF MEETING

Wednesday 12 July

Main Lecture Theatre, Queen's Medical Centre, Nottingham

14.00-21.00 Registration (at Cripps Hall)

15.30 Annual General Meeting
(Members only)

Thursday 13 July

Main Lecture Theatre Queen's Medical Centre, Nottingham

08.45 Registration desk opens

09.10 Address by the President:
Dr P. D. C. Kinmont

09.15 Guest Lecturer: Professor G. H. Elder Recent advances in the cutaneous hepatic porphyrias

09.45 N.B. Simpson, P.E. Bowden, W.J. Cunliffe and R.A. Forsner Tolerance to the anti-seborrhoeic effect of topically applied progesterone

10.05 R.A.J. Eady, C.B. Bentley-Phillips, T.M. Keahey and M.W. Greaves Cold urticaria vasculitis

10.25 P.J.A. Holt, M.G. Davies and G. Huki Pyoderma gangrenosum: clinical and investigative findings in 15 patients

10.45 G.C. Wells and Neil Smith Eosinophilic cellulitis: persistent cutaneous swelling with phagocytosis of eosinophilic debris

11.05

Coffee

11.25

P.M.Hudson, M.M.Black and
I.W.WhimsterNodular prurigo: a clinical,
biochemical and histological study

11.45

B.Bentley-Phillips

Hereditary hypotrichosis: a previously
undescribed variant

12.05

Peter D. Samman

Trachyonychia (rough nails)

12.25

J.L.Burton, R.M.M.Harman,
R.D.G. Peachey and R.P.WarinA controlled trial of azathioprine in
the treatment of pemphigoid

12.45

Lunch

14.00

Guest Lecturer: Professor
R.W.Baldwin

Prospects for tumour immunotherapy

14.35

Rona M. MacKie, Rebecca
Cochrane, John Thompson and
Sue CobbA study of IgE and RAST levels
in patients with chronic atopic
dermatitis

14.55

W.G.Reeves, Elizabeth Mitchell,
Mary Crone, B.R. Allen,
D.W.Marriott and T.B.
WallingtonImmunofluorescent and serological
findings in 110 consecutive patients
with suspected bullous skin
disorders

15.15

David A. Norris

Mycosis fungoides: lack of helper
lymphocytes in lymphocyte monocyte
interactions

15.55

Tea

16.15

D.J.Gould, G. Gowland and
W.J.CunliffeAn investigation of leukocyte function
in acne vulgaris

16.35

L.G.Millard, N.R. Rowell and
P.A.ChildPrognosis in cutaneous lupus
erythematosus

16.55

W. Fabisch

Psychiatric aspects of dermatitis
artefacta

17.15

Sam Shuster, G.H.Fisher,
E. Harris and D. BinnellThe effect of skin disease on self
image

19.00

President's Reception: Lakeside
Pavilion

Friday 14 July

Main Lecture Theatre, Queen's Medical Centre, Nottingham

08.45	Registration desk opens	
09.15	Guest Lecturer: Professor Klaus Wolff	The Langerhans cell
09.50	M.J.Embleton and M.R.Price	Detection and partial purification of melanoma-associated antigen
10.10	A.S.Breathnach, B.Martin, M.Nazzaro-Porro, S.Passi, G.Morpurgo, P.Mann and J.Cooper	Effect of dicarboxylic acids on normal human melanocytes in culture
10.30	S.S.Bleehen	Freckles induced by PUVA treatment
10.50	W.L.Morison, J.A.Ferrish and K.J.Bloch	The <i>in vivo</i> effect of UVB radiation on lymphocyte function
11.10	Coffee	
11.30	A.Finlay, S.Nicholls, S.King and R.Marks	The dry skin associated with atopic eczema
11.50	C.F.H.Vickers	Factors influencing the prognosis of infantile eczema: another ten years
12.10	Alan B. Shrank	Shoe dermatosis in children
12.30	Lunch	

Saturday 15 July

Clinical Meeting, Department of Dermatology, University Hospital, Queen's Medical Centre and Main Lecture Theatre, Medical Sciences Block, Queen's Medical Centre, Nottingham

09.30	Presentation of cases in the Department of Dermatology, University Hospital
10.30	Coffee in the foyer of the Medical Sciences Block

8

Programme of meeting

11.30

Discussion of cases in the Main
Lecture Theatre, Medical
Sciences Block

13.00

Sherry & Lunch in the
Examination Hall, Medical
Sciences Block

SUMMARIES OF PAPERS

Tolerance to the anti-seborrhoeic effect of topically applied progesterone

N.B.SIMPSON, P.E.BOWDEN, W.J.CUNLIFFE AND R.A.FORSTER

Department of Dermatology, The General Infirmary, Leeds

Acne and seborrhoea are androgen dependent; anti-androgens, such as progesterone, offer therapeutic promise in the treatment of acne.

We have, therefore, investigated the anti-androgenic effect of a twice daily applied 5% progesterone solution (in 50% ethyl alcohol) by measuring the sebum excretion rate (SER). SER was measured monthly, twice before starting treatment and three times during the treatment period.

It was not possible to assess the acne clinically as the patients, for ethical reasons, were allowed to continue on oral antibiotics during the trial.

The control group showed no significant change in SER throughout the trial. Nor was there any change in SER of males treated with progesterone. However, females treated with progesterone showed a significant decrease in SER by the end of the second month; this was most marked in females with a high initial SER (27%) ($P < 0.01$). However, at the end of the third month the SER rose towards control levels.

The lack of effect in males suggests that topically applied progesterone is incapable of overcoming the androgen control of sebaceous gland in males. The loss of effect in the third month in females suggests a shift towards alternative pathways for cutaneous androgen metabolism.

This study raises some doubt as to the possible clinical benefit of certain anti-androgens in the treatment of acne.

Cold urticaria vasculitis

R.A.J.EADY, C.B.BENTLEY-PHILLIPS, T.M.KEAHEY AND M.W.GREAVES

Institute of Dermatology, St John's Hospital for Diseases of the Skin, Homerton Grove, London E9 6BX

Vasculitis may be an important histological feature of chronic urticaria associated with systemic disease (Sissons *et al.*, 1974; Soter, Austen & Gigli, 1974). Possibly, vasculitis underlies a variety of more common urticarial reactions, but newer techniques are needed to demonstrate the vascular changes, especially when mild or in the early stages of their evolution. We found that vasculitis could be induced in the skin of several patients with essential acquired cold urticaria, by repeated cold challenge, as performed during cold-tolerance treatment (Bentley-Phillips, Kobza Black & Greaves, 1976).

Urticaria was evoked in eight patients either by immersing their arms in cold water (7–15°C) or by ice application for 5 min. This procedure was repeated every 30 min until a total of 7–15 cold applications had been completed. Biopsies were taken from urticated and oedematous skin in each subject after both single and repeated cold challenge. All specimens were processed for electron microscopy (EM) using standard methods. Material from four patients was also prepared for immunofluorescence examination (IMF).

In five patients after repeated cold challenge, histological examination of 1 µm plastic sections revealed features of early vasculitis principally affecting venules in middle and deep dermal zones and subcutaneous tissue. The endothelial cells were swollen or vacuolated and separated by gaps often containing blood cells. There was a perivascular infiltrate of neutrophils, eosinophils, monocytes and erythrocytes, with fibrin deposition. EM showed cytolytic changes in both neutrophils and eosinophils. Mast cells were variably degranulated. These vasculitic changes were not seen after single cold challenge in any of the patients, nor in the skin of two control subjects after repeated ice applications. IMF showed perivascular deposition of IgM, C₃ and fibrin in sequential biopsies from one patient, but only fibrin deposition in biopsies from the other three subjects.

The demonstration of these inflammatory changes after repeated cold challenge presumably reflects sustained injury to small cutaneous blood vessels, in a condition associated with release of potent inflammatory mediators. The IMF findings suggest that deposition of immune complexes may be important in some instances of cold urticaria vasculitis. It is of interest that the vasculitis was most pronounced in patients responding poorly to cold-tolerance treatment.

REFERENCES

- SISSONS, J.G.P., WILLIAMS, D.G., PETERS, D.K., BOULTON-JONES, J.M. & GOLDSMITH, H.J. (1974) Skin lesions, angio-oedema and hypocomplementaemia. *Lancet*, **ii**, 1350.
 SOTER, N.A., AUSTEN, K.F. & GIGLI, I. (1974) Urticaria and arthralgias as a manifestation of necrotizing angitis (vasculitis). *Journal of Investigative Dermatology*, **63**, 485.
 BENTLEY-PHILLIPS, C.B., KOBZA BLACK, A. & GREAVES, M.W. (1976) Induced tolerance in cold urticaria caused by cold-evoked histamine release. *Lancet*, **ii**, 63.

Pyoderma gangrenosum—clinical and investigative findings in 15 patients

P.J.A.HOLT, M.G.DAVIES AND G.NUKI

Department of Dermatology, Department of Rheumatology, University Hospital of Wales, Cardiff CF4 4WX

Fifteen consecutive patients with pyoderma gangrenosum (PG) have been studied during the period 1971–78. Systemic disease was found in 13 of the patients and preceded the skin disease in 10 patients by 3–25 years. Eight patients had polyarthritis; this was classical seropositive rheumatoid arthritis in two patients, but four patients had an unusual progressive erosive seronegative polyarthritis. Three patients had an IgA myeloma. Two patients only had ulcerative colitis. One patient had paroxysmal nocturnal haemoglobinuria. In only the two patients with ulcerative colitis was there a correlation between the activity of the associated disease and the onset and progression of the PG.

PG developed at the site of skin trauma in six patients. The natural history of the skin disease could be classified into two groups:

- (1) An acute, progressive course in which the ulcers rapidly enlarged until arrested by treatment.
- (2) A chronic course, in which the lesions extended slowly, and then gradually healed spontaneously.

Twelve of the patients with PG have been treated with systemic corticosteroids. Five require maintenance therapy with corticosteroids; six patients are off steroids and have been free from skin disease for between 6 months and 4 years. Four patients have died, all from complications of steroid therapy.

Serum complement levels were normal and tests for circulating immune complexes were negative. Skin histology showed no evidence of vasculitis and direct immunofluorescence of involved skin was negative for IgG, IgM, IgA and C3. No consistent abnormality of cell mediated immunity or neutrophil function was observed and HLA B27 was absent on tissue typing.

Eosinophilic cellulitis—persistent cutaneous swelling with phagocytosis of eosinophilic debris

G.C. WELLS AND N. SMITH

St John's Hospital for Diseases of the Skin, London WC2

The term recurrent granulomatous dermatitis with eosinophilia was first used in 1971 to describe four cases of an unusual dermatosis with a distinctive clinical picture and histopathology (Wells, 1971).

Early lesions show dermal oedema and dense infiltration with eosinophils. A few weeks later histiocytes dominate the infiltrate and characteristic 'flame figures' are seen scattered throughout the dermis. These consist of a central flame-like mass composed of part of a collagen bundle coated with eosinophilic debris surrounded by a palisade of large histiocytes and foreign body giant cells. This appears to be a phagocytic response to the products of disintegrating eosinophils.

We have now had experience of seven further patients who have the clinical and histological features of eosinophilic cellulitis. Persistent 'urticarial' eruptions and infiltrated erythemas were the most common cutaneous signs. The lesions frequently had an annular or circinate pattern and would persist for several weeks. A peripheral blood eosinophilia of 13–44% was present in half the cases during the active phase of the disease. No evidence of parasitic infection or vasculitis was found. In most patients the condition tended to be chronic and recurrent with eventual spontaneous resolution over the course of a year or two.

In addition to these seven cases we have observed similar histopathological changes in biopsies from four patients without the usual clinical features. Two of these patients had prurigo-like lesions, one had herpes gestationis and in the fourth a dermatophyte was cultured from the skin.

Although the histopathological features of eosinophilic cellulitis are often associated with a particular clinical picture, in some instances it appears that this reaction pattern may be triggered off by various different stimuli.

REFERENCE

- WELLS, G.C. (1971) Recurrent granulomatous dermatitis with eosinophilia. *Transactions of the St John's Hospital Dermatological Society*, **57**, 46.

Nodular prurigo—a clinical, biochemical and histological study

P.M. HUDSON, M.M. BLACK AND I.W. WHIMSTER

Department of Dermatology, St Thomas' Hospital, London SE1 7EH

Hitherto, nodular prurigo has not been studied in any detail although there have been sporadic reports of nodular prurigo associated with malabsorption. We have studied ten cases (three male and seven female) of nodular prurigo, clinically, biochemically and histologically.

Biopsies of the nodules were divided into three parts, one part for routine histology, one for vital staining using a modified methylene blue technique and one processed for electron microscopy.

The patients were investigated haematologically and biochemically for a possible underlying cause for their pruritus.

The age at onset of nodular prurigo varied widely (23–68 years). Two cases were found to have co-existent diseases which may have contributed to their pruritus; their clinical course improved when their conditions were treated. In the others the disease tended to be chronic and relapsing. None of our patients had any biochemical evidence of malabsorption and a small bowel biopsy (normal) was performed in only one patient.

Histologically the nodules showed characteristic changes with varying degrees of localized lichenification and an increase of neural material in the upper and mid dermis. The modified methylene blue nerve stain showed hyperplasia and degeneration of unmyelinated nerve fibres. Electron microscopically it was seen that each Schwann cell encircled numerous axons (20–30) of differing diameter. The neural proliferation is thought to be a reactive phenomenon. These changes differ from those seen in hypertrophic lichen planus and lichen simplex chronicus.

Although none of our patients showed any evidence of malabsorption, we feel that in an otherwise intractable condition every effort should be made to eliminate a possible underlying cause for pruritus.

Hereditary hypotrichosis: a previously undescribed variant

B. BENTLEY-PHILLIPS

Dermatology Clinic, King Edward VIII Hospital, Congella, Durban, South Africa

An extremely rare type of hereditary hypotrichosis with an unusual natural history and clinical features is described in a Caucasian family, originally of British stock and now living in South Africa. In a pedigree of four generations, with a total of 25 members, 11 are affected. The mode of inheritance is autosomal dominant with no sex limitation.

In all cases the hair loss commences during the school years and progresses gradually to almost complete baldness of the scalp leaving only a few wispy hairs. The eyebrows and eyelashes disappear and body hair becomes sparse. The clinical pattern in these cases differs from that of other distinct varieties of hereditary hypotrichosis, for example Marie Unna (1925) where the hair is sparse or absent at birth and then becomes apparently normal, other syndromes with associated ectodermal and skeletal abnormalities and hypotrichosis simplex limited to scalp (Toribio & Quinones, 1974).

In 1924, Jeanselme and Rime described a family of 5 generations including 35 individuals of whom 14 were affected, showing a somewhat clinical picture to the family under discussion but exhibiting several distinct differences.

The pedigree together with serial photographs of 3 members of the family ranging from childhood to adult life will be shown. Results of other investigations which include aspects of histology, biochemistry, light and scanning electron microscopy, immunology and chromosomal studies will be presented.

REFERENCES

- JEANSELME & RIME (1924) Un cas d'alopécie congénitale familiale. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **31**, 79.
 TORIBIO, J. & QUINONES, P.A. (1974) Hereditary hypotrichosis simplex of the scalp. *British Journal of Dermatology*, **91**, 687.
 UNNA, M. (1925) Über Hypotrichosis Congenita Hereditaria. *Dermatologische Wochenschrift*, **81**, 1167.

Trachyonychia (rough nails)

P.D.SAMMAN

The Westminster Hospital, London

The term trachyonychia was coined by Alkiewicz in 1950 to record cases of rough nails. It was taken up by Achten and Wanet-Ronard in a classification of nail dystrophies of known and unknown causes. Hazelrigg, Duncan & Jarratt recently named some of these cases the twenty-nail dystrophy of childhood. Baran & Dupré in a letter to the *Archives of Dermatology* pointed out that some cases are probably due to alopecia areata and when the whole nail is not involved lichen planus or psoriasis may be responsible.

If cases due to psoriasis and alopecia areata are excluded, we are left with an assortment of cases which fall into two groups:

(1) Twenty-nail dystrophy of childhood: In this group ridging is the chief characteristic. Almost all cases are confined to childhood and very gradually improve with age.

(2) Severe nail dystrophy: The changes here are even more severe and excess ridging is not a particular feature. The nails are discoloured, rough and may be thick or thin, and one or more will probably show koilonychia. One or more nails are also usually spared. Again most cases are seen in childhood. The severe changes seen in a very few cases of alopecia areata mimic this picture very closely.

(Numerous photographs will be shown.)

REFERENCES

- ACHTEN, G. & WANET-ROUARD, J. (1974) Atrophie unguale et trachyonychie. *Archives belges de Dermatologie et de Syphiligraphie*, **30**, 201.
 ALKIEWIEZ, J. (1950) Trachyonychie. *Annales de dermatologie et de syphiligraphie*, **10**, 136.
 BARAN, R. & DUPRÉ, A. (1977) Vertical striated sandpaper nails. *Archives of Dermatology*, **113**, 1613.
 HAZELRIGG, D.E., DUNCAN, W.C. & JARRATT, M. (1977) Twenty-nail dystrophy of childhood. *Archives of Dermatology*, **113**, 73.

A controlled trial of azathioprine in the treatment of pemphigoid

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Azathioprine will suppress pemphigoid (Burton & Greaves, 1974) but the long-term side-effects of immunosuppression in elderly patients are uncertain. We therefore performed a prospective controlled trial of azathioprine (2.5 mg/kg body weight) with prednisone (dose adjusted as necessary) versus prednisone alone in the treatment of 25 consecutive patients with pemphigoid (confirmed by histology and direct immunofluorescence). Each patient was carefully followed up for 3 years and the results were as follows:

	Azathioprine + prednisone	Prednisone alone
No. of patients	12	13
Sex	6 M and 6 F	3 M and 10 F
Mean age at onset	75.6 years	74.1 years
Mean dose of prednisone (mg. p.a.)		
Year 1	2980	4362
Year 2	400	1650
Year 3	308	720
Outcome after 3 years		
Deaths	3	4
Cause of deaths	1. C.V.A. 2. C.V.A. 3. Cong. cardiac failure	1. Bronchopneumonia 2. Bleeding from peptic ulcer 3. Mesenteric thrombosis 4. C.V.A.
In remission	7	3
Well, but still needing therapy	2	5
Withdrawn from trial due to side effects of therapy	0	1 (due to hypertension)

We conclude that the use of maintenance azathioprine therapy following initial control of pemphigoid with prednisone improved the prognosis over a 3 year period. The total dose of steroids required for maintenance was significantly reduced ($P < 0.01$) and the risk of serious side-effects from steroid therapy was correspondingly decreased. Some immunosuppressive drugs such as cyclophosphamide have been implicated in the development of malignancy (Knowlson, 1977), but none of our patients developed malignancy and side effects attributable to azathioprine were minimal.

REFERENCES

- BURTON, J.L. & GREAVES, M.L.J. (1974) Azathioprine for pemphigus and pemphigoid—a four year follow-up. *British Journal of Dermatology*, **91**, 103.
KNOWLSON, P.A. (1977) Cyclophosphamide and malignancy. *Lancet*, **ii**, 457.

A study of IgE and RAST levels in patients with chronic atopic dermatitis

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Ten young adults with chronic atopic dermatitis have been studied over a period of 1 year during exacerbations and remissions of their cutaneous lesions. Serial estimations of total serum IgE and specific IgE by the radio-allergosorbent test (RAST) have been carried out with the aim of determining: (1) whether or not there is a difference in IgE levels between patients with 'pure' cutaneous atopy and those with additional respiratory symptoms; (2) whether or not total serum IgE levels change with the patient's clinical state; and (3) the relationship between the patient's clinical history and positive results obtained by the RAST technique.

All ten patients showed continuously elevated total serum IgE levels and six of the seven in the study with pure cutaneous atopy had elevated levels similar to those found in the remaining three with mixed cutaneous and respiratory symptoms of atopy. No clear pattern of variation of serum IgE levels was observed in patients with exacerbation of cutaneous lesions and even patients with periods of prolonged remission (9 months) did not show a significant drop in total serum IgE.

A clear relationship between the patient's history and positive results by the RAST technique was obtained in four instances. Positive results to specific allergens were obtained in the absence of a positive history but in no case was a clear clinical history of an allergic response accompanied by a negative result to the appropriate antigen.

Immunofluorescent and serological findings in 110 patients with suspected bullous skin disorders

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One hundred and ten patients with suspected bullous skin disease were investigated immunologically. Clinically they consisted of 63 with overt bullae and 47 without. The latter were mostly characterized by the presence of 'itchy papules' and are designated as such. Most biopsies were taken from non-lesional skin and examined by immunofluorescence for the presence of IgG, IgA, IgM, C3, C1q and fibrinogen.

In the **bullous** category biopsies were usually performed to seek confirmation of the clinical diagnosis. Four patterns of deposition were recognized:

(a) *Inter-cellular epidermal IgG ± C3*. All nine patients had a clinical diagnosis of pemphigus. Six showed deposition of C3 as well as IgG and 7/9 sera contained skin reactive antibody (SRA) of pemphigus type and IgG class.

(b) *Granular sub-epidermal IgA ± C3*. All 13 cases were diagnosed as dermatitis herpetiformis and all showed a good response to dapsone. 10/13 biopsies contained C3 in addition to IgA and of nine sera tested all lacked SRA of either type.

(c) *Linear C3 ± IgG at the epidermal-dermal junction.* Of the 29 patients with both materials present, 27 had a clinical diagnosis of pemphigoid. Seventeen of these 28 sera contained SRA of pemphigoid type and IgG class. Two patients had cicatricial pemphigoid and herpes gestationis respectively and lacked SRA. Six other patients lacked IgG in their biopsies or SRA. Their clinical diagnoses were: herpes gestationis (3), pemphigoid (2) and epidermolysis bullosa acquisita (1).

(d) The findings in a *miscellaneous* group of six patients will be described.

The 47 patients in the **itchy papular** category were usually biopsied to seek diagnostic information in the presence of clinical uncertainty. Several patterns were identified:

(e) Eleven patients showed *granular sub-epidermal IgA and C3*. Nine responded well to dapsone, one to a gluten-free diet and one received neither.

(f) Six patients showed *sub-epidermal C3 alone*. Of two treated with dapsone, one responded well.

The six patterns seen in the remaining 30 patients will be described.

Of the 43 sera tested in the 'itchy papular' category none contained SRA.

Mycosis fungoides: lack of helper lymphocytes in lymphocyte-monocyte interactions

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This study shows that the abnormal monocyte chemotaxis found in patients with mycosis fungoides is due to lack of lymphocyte helper function and not an intrinsic monocyte defect or inhibitor substance. Mononuclear preparations from 10 patients and 10 controls were prepared by Ficoll-Hypaque separation, and pure lymphocyte preparations were obtained by glass bead column separation. Mononuclear cells and purified lymphocytes from normals and mycosis fungoides patients were added in Boyden chambers and migration through nucleopore filters in response to endotoxin activated serum was quantified.

Monocyte source	Lymphocyte source	Cells/20 oil fields
M.F.	M.F.	119.2 ± 57
	Control	337 ± 114
Control	M.F.F.	320 ± 60.1
	Control	324 ± 42.1

} P=0.05

Lymphocytes from controls significantly improved the function of monocytes from M.F. patients, but no inhibition of control function with the addition of M.F. lymphocytes could be seen. In addition, assays for dissociable cell directed chemotactic inhibitor, or for soluble inhibitor or helper factors produced in culture were negative. There were no differences in the levels of endogenous chemotactic factors or in the production of lymphocyte derived chemotactic factor (LDCF) in culture between controls and M.F. patients. Thus, a functional deficiency in lymphocytes may be the principal cause of the decreased monocyte chemotaxis seen *in vitro*. The demonstration that functional alterations of peripheral blood lymphocytes in early M.F. can affect the function of other peripheral blood cell types may indeed be directly related to the accumulation of monocytes and lymphocytes in the tissue infiltrates of M.F.

An investigation of leukocyte function in acne vulgaris

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Mediators of inflammation are an aspect of acne pathogenesis which have received scant attention. We have, therefore, measured two parameters of leukocyte function in 78 patients with acne vulgaris. We estimated the intracellular killing power of polymorphonuclear leukocytes (PMN) by the nitro-blue tetrazolium test (N.B.T.) and the *in vitro* chemotactic response to casein of PMN and monocytes. The correlation between clinical acne severity using a subjective ranking scale and these variables was determined using Spearman's Rank correlation test. There was no correlation between acne and the N.B.T. test ($R_s = 0.0328$; ns). Failure to demonstrate enhanced intracellular killing by the N.B.T. test probably reflects the well-documented insensitivity of the test when used to measure changes in neutrophil function in well-localized infection.

A significant positive correlation existed between acne grade and PMN chemotaxis ($R_s = 0.5568$; $P = 0.001$) and between acne grade and casein stimulated random mobility ($R_s = 0.3148$; $P = 0.004$) for neutrophils but not for monocytes, whose random unstimulated mobility ($R_s = 0.2260$; $P = 0.036$) only was increased.

The increased chemotaxis demonstrated by neutrophils from patients with acne to casein or standard chemotactic agent suggests that these cells exist in a state of enhanced responsiveness compared to those patients without acne.

We believe these findings are indirect evidence in support of a stimulation of neutrophil responsiveness to infection in patients with acne vulgaris. They may also explain why tetracycline, a known inhibitor of neutrophil chemotaxis, is so efficient in this condition.

Prognosis in cutaneous lupus erythematosus

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One hundred and ten patients (70 F, 40 M) with cutaneous lupus erythematosus (LE), who were investigated during 1958-62, were reviewed in 1975-77. The disease remitted completely in 48 (43.6%). Six patients (5.5%), (5 F, 1 M) developed systemic lupus erythematosus (SLE). Long duration of disease and lack of remission was significantly related to Raynaud's phenomenon, scalp involvement and chilblain type lesions. The development of SLE was not related to the distribution or extent of cutaneous lesions but was commoner in those patients with lesions on the hands and feet.

Haematological and immunological abnormalities are found in more than half the patients with cutaneous LE but the same abnormalities do not usually persist. Immunoglobulin analysis is not a good indication of activity. Constant laboratory abnormalities were present from the beginning in all six patients who eventually developed SLE, but did not differentiate this group clearly from patients with disseminated cutaneous LE alone.

Homogeneous or speckled pattern antinuclear factor in a titre of $> 1:50$ was consistently found in all patients developing SLE but three patients with chilblain LE and one with inactive disseminated

disease showed these patterns also. Antinuclear factor in a titre of $< 1:50$ did not indicate potentially prolonged cutaneous or systemic disease.

Squamous carcinomatous change occurred in the cutaneous lesions of four males and one female (4.5%). The two patients with scalp carcinomata died of multiple metastases within 18 months.

Psychiatric aspects of dermatitis artefacta

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Fifty patients were investigated psychiatrically over several years, and 26 of these were also seen independently by a clinical psychologist.

The psychological investigations suggest a personality structure characterized by inward looking self-centred attitudes. The patients' interests are generally limited and constricted. Warm-hearted outgoing personalities enjoying well-established satisfactory relationships with others are rare.

Psychiatric investigations suggest a background of emotional disturbances which, during the formative years, are often due to adverse home circumstances, illness, bereavement or sibling rivalry. In later years matrimonial and work problems play a similar role. The results are often feelings of isolation and insecurity.

The onset of dermatitis artefacta is very often related to definable precipitating events varying from school problems to sexual, matrimonial, or work difficulties and ill health according to age and circumstances. The skin lesions, visible in part at least, can be understood as an attempt at non-verbal communication by persons who find it difficult to say what they feel.

Therapeutic approaches and prognosis will be discussed in some detail.

The effect of skin disease on self image

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Because of the instinctual aversion to skin disease and its function as a major organ of social and sexual communication we predicted that skin disease would lead to psychological disturbance particularly of self image. Acne was studied in relation to severity (grades 1-4) as this gave an in-group control, eczema and psoriasis were also studied, and two control groups. The method used was a grid of nine schematic faces previously rated for the attributes of age, happiness, intelligence, good looks and likeableness. The patients and controls were asked to select the faces for the attributes of: like me; what I'd like to be like; me in 10 years time; me 10 years ago; like someone I like; like someone I dislike; what other people think I'm like; likeable; good looking. Each attribute was then correlated with each other for each group of patients and controls. The results showed a strong statistical correlation between 'like me' and 'desirable' characteristics such as 'likeable' and 'good looking' in control subjects. In acne as severity increased there was a progressive, dramatic and highly significant decrease in this correlation with a decline both in self image and belief in what others thought of it;

at the same time there was a significant correlation with attributes of 'someone I dislike'. This was more marked in females. Eczema and psoriasis showed a less severe damage to self image which may have been related to the different site of involvement. Thus there is a deterioration in self image in patients suffering from certain skin diseases which is related to severity, sex and site of disease. It is considered that this effect of skin disease on the mind is the likely basis of the association of skin and mental disturbance and not the converse.

Detection and partial purification of melanoma-associated antigen

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Sera from melanoma patients, patients with other types of cancer and normal healthy individuals were screened for antibody reactivity against a long term cultured melanoma cell line, NKI-4, by membrane immunofluorescence. About 7% of melanoma sera were reactive against this cell line, and a similar level of positive reactions was observed with sera from patients with unrelated tumours. In comparison only about 2% of normal sera were positive.

Sera reactive against NKI-4 cells were tested against a panel of cultured cells and it was found that the melanoma sera were positive against between two and four of eight melanoma lines, but NKI-4 positive sera from non-melanoma donors were negative against other melanoma cells. Also, reactivity by the melanoma patients' sera against cells derived from other tumour types was rare, suggesting degree of melanoma-associated specificity.

Antibody in melanoma sera reacting with NKI-4 cells could be removed by absorption with a soluble 3M KCl extract of melanoma tissue, but comparable extracts prepared from normal skin, embryos or carcinomas of breast, colon or lung did not absorb this antibody. The absorbing activity of the melanoma extract could be removed on an immunoadsorbent column prepared from a positive patient's serum, and eluted with 3M NaSCN. The eluted material represented less than 10% of the original KCl extract on a protein basis but was more efficient at antibody absorption, indicating partial purification of antigen activity.

Effect of dicarboxylic acids on normal human melanocytes in culture§

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Since dicarboxylic acids are competitive inhibitors of tyrosinase, and effective in treatment of hyper-pigmentary disorders, probably due to cytotoxic effect on abnormal melanocytes, it is of interest to examine their effect on normal melanocytes in culture.

§Supported by the Wellcome Trust.

Azelaic (C9) and dodecandioic (C12) acids, to final concentrations of 100 and 200 $\mu\text{g/ml}$, were added to dispersed cultures of epidermal cells grown in Eagle's MEM with HEPES salts, glutamine, and 18% fetal calf serum. At 7, 10, 15 and 20 days, control, and experimental cultures were processed for electron microscopy.

Results were essentially similar with C9 and C12 acids. At 7, 10 and 15 days, melanocytes of experimental cultures exhibited significantly increased melanogenic activity compared with controls. Disintegrating melanosomes were present in cells of both, but more numerous in experimental ones. At 20 days, control and experimental melanocytes were essentially similar, with poorly melanized melanosomes, but healthy.

We conclude that dicarboxylic acids have an initial stimulatory effect on melanocytes of the original culture generation and that this may result in destruction of melanosomes. Their presence in the medium at present concentrations does not prevent growth of, nor cause damage to, a second generation of cells up to 20 days.

Freckles induced by PUVA treatment

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A profuse freckling of the skin has been observed in three patients having systemic photochemotherapy using 8-methoxypsoralen and long wave ultraviolet light (UVA). This side effect of PUVA treatment occurs not infrequently in fair skinned individuals.

Light and electron microscope studies have been carried out on biopsies taken from these stellate freckles and from paler areas of skin. Similar studies have been performed on biopsies from two freckled, fair-skinned and red-haired individuals and from one freckled patient receiving UVA light alone.

No significant histological, histochemical and ultrastructural differences were found between the PUVA induced freckles occurring in normal fair-skinned individuals. The melanocytes in the freckled skin were larger, more dendritic and more dopa positive than in the paler skin. They contained many melanosomes in various stages of development, mostly ellipsoidal in shape with a filamentous internal architecture and a mean transverse diameter of $0.14 \mu\text{m}$. A variable number of spherical, smaller and granular melanosomes were observed. No abnormal melanocytes containing abortive abnormal melanosomal forms were found.

These studies indicate that PUVA induced freckles are banal and become unmasked by the therapy. Previous studies have indicated (Breathnach, 1959; Breathnach & Wyllie, 1964) that in the skin of freckled individuals there are two types of melanocytes with different capacities for melanogenesis.

REFERENCES

- BREATHNACH, A.S. (1959) An attempt to induce pigment spread on freckled human skin. *Journal of Investigative Dermatology*, **33**, 193.
BREATHNACH, A.S. & WYLLIE, L.M. (1964) Electron microscopy of melanocytes and melanosomes in freckled human epidermis. *Journal of Investigative Dermatology*, **42**, 389.

The *in vivo* effect of UVB radiation on lymphocyte function

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The effect of whole-body exposure to UVB radiation on the distribution and function of peripheral blood lymphocytes was investigated in ten normal volunteer subjects from whom informed consent had been obtained. The MED of UVB and diurnal variation in lymphocyte distribution and function were first studied in each subject. A fraction or multiple of the MED was then given as a whole-body exposure resulting in responses ranging from no erythema to fiery redness with areas of oedema. Enumeration of T and B lymphocytes and measurement of the incorporation of tritiated thymidine into DNA following stimulation by phytohaemagglutinin were performed serially during the 72 h after exposure. Erythemogenic doses of UVB resulted in a significant depression of the proportion of T lymphocytes, a minimal depression of B lymphocytes and a decreased response to PHA stimulation. These effects reached a maximum within the first 12 h after exposure and all reverted to normal within 72 h after irradiation. Changes were dose-related, and exposures resulting in minimal or no erythema were not associated with significant changes. These findings suggest that certain doses of UVB radiation can alter lymphocyte distribution and function but that the effect is short-lived and reversible.

The dry skin associated with atopic eczema

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Many patients with atopic eczema have dry and scaling skin in the non-eczematous areas. The aim of this study has been to characterize this condition and its relationship to autosomal dominant ichthyosis (ADI) and the eczematous rash that also affects these patients.

We have studied the outer aspect of the upper arm (a non-eczematous area) in 14 young adults with atopic eczema and dry skin, and normal controls.

Corneocytes were collected using a modified scrub technique (Nicholls & Marks, 1971). Measurements of average corneocyte diameter with a graticule showed the cells from the eczema group to be significantly smaller than normal (dry skin = $33.5 \pm 1.08 \mu\text{m}$; normal = $38.32 \pm 1.18 \mu\text{m}$; $P < 0.001$). Total cell counts tended to be higher in the eczema group with cell clumping also apparent (dry skin $66.5 \pm 18.9 \text{ cells/mm}^2$, normal $57.3 \pm 10.9 \text{ cells/mm}^2$).

Cohesography (Nicholls & Marks, 1977) has given normal results in most subjects studied, but as expected was abnormally high in two very 'dry' cases (150 g; normal < 100 g). Using the skin surface replica technique and surfometer (Nicholls, King & Marks, 1978) we have quantified the increased roughness of the dry skin and found that it provided a useful method for assessing the results of therapy. Macrophotographs indicated differences in the surface patterning between atopic dry skin and ADI. Using light microscopy we have found no significant differences in mean keratinocyte height, basal/granular cell ratios, mean epidermal thickness or the number of cell layers in the stratum corneum. The eczematous eruption did not occur at sites of maximal dryness. It appeared

clinically that the severity of the dryness and the eczematous eruption fluctuated independently. The dryness was inconstantly present in family members. It is suggested that:

- (1) There is no direct pathogenetic relationship between the dry skin and the eczema.
- (2) The atopic dry skin differs from ADI.

REFERENCES

- NICHOLLS, S. & MARKS, R. (1977) Novel techniques for the estimation of intracorneal cohesion *in vivo*. *British Journal of Dermatology*, **96**, 595.
- NICHOLLS, S., KING, C.S.A. & MARKS, R. (1978) Morphological and quantification assessment of physical changes in the horny layer in ichthyosis. In: *The Ichthyoses* (Ed. by R. Marks and P.J. Dykes). MTP Press, Lancaster.

Factors influencing the prognosis of infantile eczema—another 10 years

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This study, a preliminary report of which was presented at a meeting of the British Association of Dermatologists in Sheffield, in 1970, now includes 2000 children and covers a period of 20 years. Certain outstanding features of the study will be described.

Adverse factors affecting prognosis are now clear-cut: these include late onset (after 15 months of age), the so-called reversed pattern, discoid eczema and parental discord and social problems.

Favourable factors include early onset (before 6 months of age), the so-called seborrhoeic pattern and the effect of the persistent use of topical corticosteroids and nocturnal antihistamines.

The recovery rate at 15 years of age is approximately 93% and certain factors can now be shown to be important in recovery.

It is not possible as yet to determine accurately the relapse rate at puberty.

Shoe dermatosis in children

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With the change from the use of leather to plastics and rubber for the soles of children's shoes, a characteristic dermatosis has appeared on children's soles and toes. It consists of a fairly well-defined coarsening in the texture of the skin of the anterior part of the sole and plantar aspect of the toes sparing the toewebbs. It may be worse in the summer and cracks may develop, but no rash occurs elsewhere. There is no personal history of atopy or a family history of atopy, ichthyosis or psoriasis. Patch testing to a variety of chemicals and materials from the shoes has proved unhelpful.

Of 100 patients of all ages with foot eczema seen in hospital clinics in Shropshire since 1967, 38 have been children with this complaint, 33 presenting since 1974. Half the affected children were boys with an average age of onset of 6.7 years (range 2–12), whilst the average age of onset for the girls was 7.4 years (range 2–15). A follow-up study will be reported.

Several causes are postulated. The withdrawal of leather-soled children's shoes is considered to be the crucial factor, because it coincides with the sudden rise in the incidence of the dermatosis.

POSTER DEMONSTRATIONS FOR THE 1978 SUMMER B.A.D. MEETING

(1) A syndrome of lumpy scalp, odd ears and rudimentary nipples

A.FINLAY AND R.MARKS*

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(2) The effect of X-rays on the chromosomes of patients with ataxia telangiectasia: an aid to diagnosis

A.M.R.TAYLOR

Department of Cancer Studies, The Medical School, Birmingham B15 2TJ

(3) The histology of vitiligo

DR S.S.BLEEHEEN

University of Sheffield Academic Division of Medicine, Sub-Department of Dermatology, The Halamshire Hospital, Sheffield S10 2JF

(4) Infestation by *Dermatobia hominis*, the human bot fly

P.J.HARE AND MARY BUNNEY

University Department of Dermatology, The Royal Infirmary, Edinburgh EH3 9YW

(5) Detection of antibodies against melanoma cells by radio-isotopic methods

M.J.EMBLETON AND M.R.PRICE

Cancer Research Campaign Laboratories, University of Nottingham

(6) Bazex syndrome

D.J.GOULD, D.J.BARKER AND W.J.CUNLIFFE

Department of Dermatology, The General Infirmary, Leeds

(7) The prevalence of acne vulgaris in late adolescence and adult life

D.J.GOULD AND W.J.CUNLIFFE

Department of Dermatology, The General Infirmary, Leeds

(8) Skin reactions caused by vitamin K in patients with liver disease

A.W.BULLEN*, J.P.MILLER†, W.J.CUNLIFFE‡, M.S.LOSOWSKY*

**Department of Medicine, St James' Hospital, Leeds, †Department of Medicine, Withington Hospital, Manchester, and ‡Department of Dermatology, The General Infirmary, Leeds*

(9) Effect of dicarboxylic acids on lentigo maligna

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CASE REPORTS

(1) Superficial fatty naevus

DR M.J.CHEESBROUGH FOR DR P.D.C.KINMON

General Hospital, Nottingham

E.M., female, aged 26 years. Hospital case no. 13884.

History. She has had a lesion on her lower back all her life. This has been a cosmetic problem only.

Examination. A lobulated, yellowish nodule on the right buttock and natal cleft. The surface is smooth and the lesion is not tender.

Histology. This shows lobules of fat in the reticular dermis, consistent with a superficial fatty naevus (7219/76).



FIGURE 1. Superficial fatty naevus on buttock.

(2) Ichthyosis hystrix

DR B.R.ALLEN

General Hospital, Newark

D.P., female, aged 5 years. Hospital case no. 63862.

History. She was first seen in September 1976 complaining of recurrent blisters of 2-3 months duration. These were probably due to papular urticaria and did not reappear when they got rid of their dog. Ichthyosis had been present since birth but there were no previous episodes of blistering.

Past illnesses. Nil relevant.

Family history. Her mother (who accompanies her today) is similarly affected as is her maternal grandfather. A brother, who died as a cot death, had eczema and a living boy is unaffected.

Examination. She has a verrucose pigmented ichthyosis most marked around her waist and on the flexor and extensor aspects of her joints. There are no blisters.

Treatment. Her mother's lesions respond extremely well to 10% salicylic acid in soft paraffin applied on alternate days.

Investigations. Skin biopsy (8763/76) taken from her mother's antecubital fossa: 'The epidermis shows papillomatosis with marked hyperkeratosis. Small focal areas of the upper prickly cell layer show loss of cell wall boundaries but there are no microvesicles. The upper dermis has a scanty, mainly perivascular infiltrate. The overall appearances are similar to naevus verrucosus' (Dr P.J. Smith).

Comment. The autosomal dominant inheritance, clinical picture and histology fit with a diagnosis of ichthyosis hystrix.

(3) Acquired ichthyosis and unclassified lymphoma

DR M.J.CHEESBROUGH FOR DR P.D.C.KINMONT

General Hospital, Nottingham

M.C., male, aged 45 years. Hospital case no. 74343.

History. This man presented in 1964 with a 2 year eczematous eruption starting on the ankles and forearms. In 1967 he developed poikiloderma vasculare atrophicum in the right axilla. This appeared on the left foot in 1969 when ichthyosis and pigmentation were noted on the legs. Histology of an enlarged lymph gland in the right groin, biopsied in 1970, was reported as Hodgkin's disease. He was given radiotherapy to the groin in 1971. Subsequent reappraisal of the histology by Dr Wilson Jones in 1972 and Dr Tony Marshall of the London Hospital in 1973 favoured benign dermatopathic lymphadenopathy. The poikiloderma has spread and pigmentation increased since 1970. By 1974 the ichthyosis was widespread. He has never had skin tumours but there have been areas of infiltration and large subcutaneous swellings treated by radiotherapy. He had one episode of haemoptysis in 1975 and chest X-rays showed linear and nodular markings interpreted as Hodgkin's disease. In 1977 he was investigated for right sided headache and pains in the shoulders and thoracic spine. Whilst under investigation he developed acute spinal cord compression at T1 and at operation a laminectomy was performed in August 1977; an extradural tumour was partially removed and this was followed by radiotherapy to C6 to T1 and chemotherapy with C.H.O.P. (cyclophosphamide, doxorubicin, vincristine and prednisolone).

Examination. There is extensive pigmentation and ichthyosis. Both axillae and the right elbow flexures show poikiloderma. There is a large bi-lobed subcutaneous tumour in the right loin. The scalp shows scarring alopecia on the crown and a small nodule on the right. There are enlarged glands in the left axilla and both groins.

Treatment. Since 1971 he has had nine courses of radiotherapy to skin infiltration and lymph nodes. He had three courses of chemotherapy in 1974, six courses in 1975 and one in 1977.



FIGURE 1. Legs showing acquired ichthyosis and subcutaneous nodules.

FIGURE 2. Right buttock showing poikiloderma.

Investigations. He has had six skin biopsies up to 1973 all of which have been thought to be a malignant lymphoma; views have differed on the type. The extradural mass removed in 1977 was thought to be a non-Hodgkin's lymphoma. The following have been normal in 1977—I.V.P., liver and spleen scan, bone marrow. Bone X-rays and scan in 1977 showed 'Lesions in both parietal bones, both scapulae, C7 and transverse processes of C6 and T1'.

Comment. He initially presented with a premycotic eruption and later developed a systemic lymphoma and acquired ichthyosis. Pathologists are unable to classify the lymphoma.

(4) Telangiectasia macularis perstans

DR B.R.ALLEN

General Hospital, Nottingham

E.B., male aged 66 years. Hospital case no. 118747.

History. This man was referred by Dr M. Espir of the Neurology Department whom he had consulted because of numbness of his leg following a road traffic accident. There were no abnormal neurological signs but skin changes were noted. These had given rise to no symptoms, but had appeared and slowly extended during the preceding 7 years.

Past history. He has hay fever due to pollen. There is also a history of allergy to porridge, ground nuts and coconuts since early childhood. In 1969 he had what appears to have been an anaphylactic reaction to coconut. One episode of industrial dermatitis is recorded.



FIGURE 1.

Family history. None of his relatives has suffered from skin disease.

Examination. There are widespread sheets of telangiectasia and pigmentation with a mainly central distribution. Splenomegaly has also been noted but there is no lymphadenopathy or liver enlargement.

Investigation. Haemoglobin—13.3 g/100 ml, W.B.C.—6000/mm³, normal differential. Bone marrow: there is a marked increase in the number of mast cells, approximately 5%. The marrow also contained a small number of atypical nucleolated cells. Skeletal survey shows abnormal features which may be associated with his condition. Further studies have been arranged.

IgE—30 units/ml.

Skin biopsy (6389): 'There is a marked perivascular infiltrate of chronic inflammatory cells including lymphocytes and a significant number of mast cells' (Dr P.D.James).

Comment. The late onset and progressive nature of his lesions, together with the evident bone marrow and possibly bony infiltration raise the question as to whether his condition is completely benign.

(5) Dyskeratosis congenita

DR M.J.CHEESBROUGH FOR DR P.D.C.KINMONT

Children's Hospital, Nottingham

M.M., male aged 12 years. Hospital case no. 153044 (Children's).

History. This boy came to the Eye Hospital in 1972 with photophobia and has been attending since because of troublesome keratoconjunctivitis. In 1974 he noticed increasing pigmentation of his neck and shortly afterwards he developed splitting of the nails and paronychia. He is not anhidrotic and his feet sweat excessively. Physical and mental development have been normal.

Family history. There is no consanguinity between his parents. Three of his mother's five brothers have been affected and two have died, one of a mucin secreting carcinoma of the pancreas and the other probably of leukaemia. Also, his mother's aunt has a son aged 24 who is affected. There are no known affected females. He has three normal stepbrothers who have the same mother.

Examination. At times the eyes are almost closed because of severe photophobia. The skin shows reticulate and dappled pigmentation with atrophy around the neck, front of the chest and outer aspects of the arms and legs. There are white streaks and plaques on the buccal mucosae. The teeth are normal. His nails show terminal onycholysis, longitudinal ridging and a pterygium of the left index finger. There is mild atrophy of the ridge pattern of the finger tips. The ear drums are hyper-lucent. Hair and scalp are normal as is systemic examination.

Investigations. Nail clippings were negative for fungus. Urine amino acid chromatography—normal.

Histology. Skin from the neck (936/78) shows 'Focal epidermal atrophy with distortion and apparent destruction of the basal layer. Underlying the epidermis is a mononuclear infiltrate with prominent melanophores and a number of hyaline structures resembling those found in lichen planus. Free nuclear debris is seen. Skin appendages and dermis are normal' (Dr A.M.MacKay).

Comment. Dyskeratosis congenita is transmitted by sex-linked recessive inheritance and is associated with malignancy as in his uncle.

Please do not discuss the family history with the patient or family.

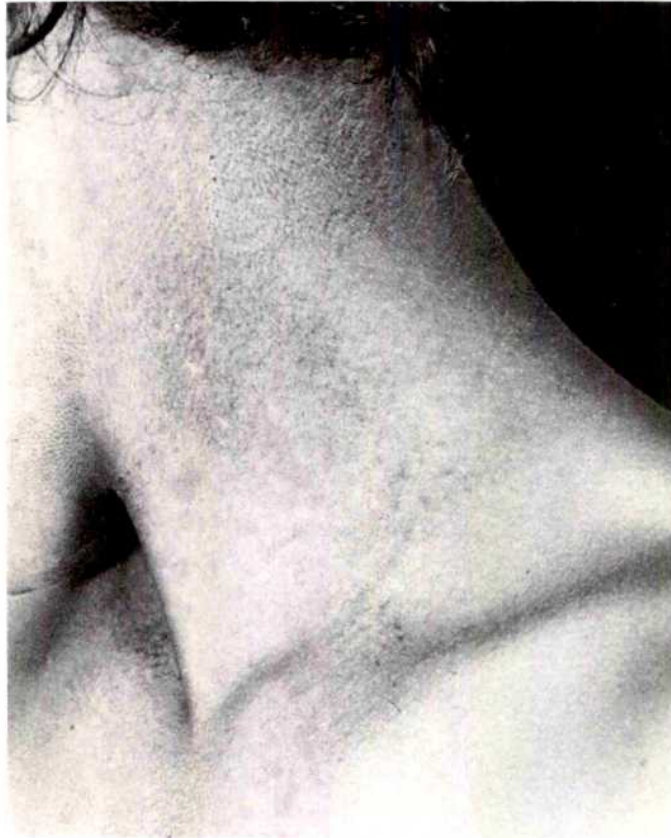


FIGURE 1. Neck showing reticulate pigmentation.

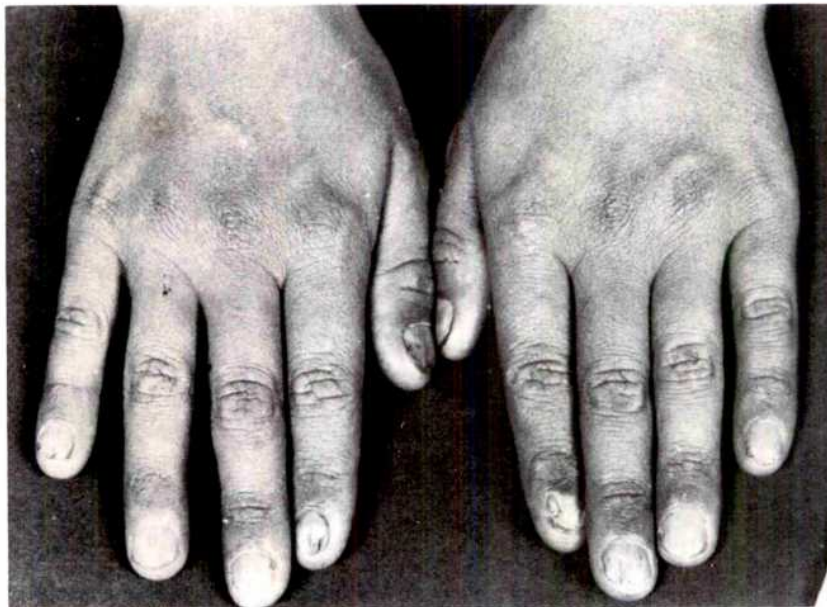


FIGURE 2. Hands showing nail dystrophy.

(6) Dyskeratosis congenita

DR J.M.GARTSIDE

Derbyshire Royal Infirmary, Derby

D.G., male, aged 31 years. Hospital case no. 17-71-92.

History. He has had gross nail dystrophy of fingers and toes since the age of 11. In addition he complains of recurrent inflammation of the eyes. There is no family history, his general health is good and he is on no drugs. He works as an electrician.

Examination. There is gross dystrophy of all nails. His palms and soles are hyperkeratotic. There is reticulate erythema with some atrophy especially of the light exposed areas of the face and arms. He has periodic inflammation of the conjunctivae and there are keratotic lesions of the buccal mucosa.

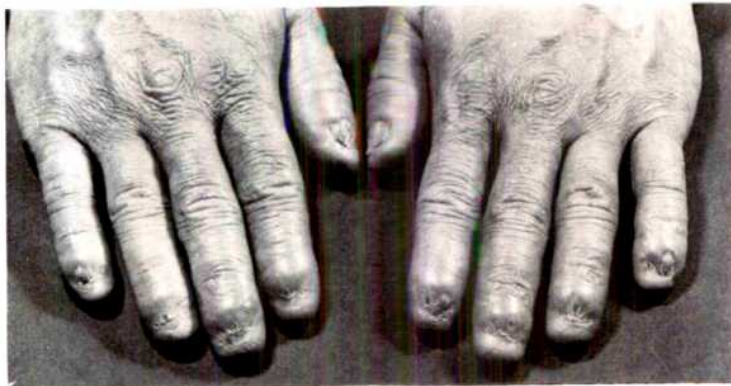


FIGURE 1. Hands showing nail dystrophy.

Investigations. Biopsy from arm shows mild changes only. There is some hyperkeratosis, sparse perivascular infiltrate and some dilation of the upper dermal vessels. Immunofluorescence—changes suggestive of mild vasculitis.

Comment. This patient shows features of the triad of signs typical of this condition; severe nail dystrophy, atrophic skin changes and mucous membrane lesions.

(7) Maffucci's syndrome

DR B.R.ALLEN

General Hospital, Nottingham

W.H., male aged 58 years. Hospital case no. 103783.

History. He first came under observation in 1941 whilst in the Army, giving a 5 year history of numbness and blueness along the ulnar border of his left hand. At about the same time exostoses were noted on the middle phalanges of the left hand.

It was thought that there may have been pressure on the arteries in his neck and an exploration of the posterior triangle was carried out. This revealed nothing abnormal and a sympathectomy resulted in an improvement in his symptoms. In 1968 he attended Dr D.I. McCallum because of a keratotic lesion on his left palm and the multiple exostoses were noted (see photographs).

Since this time there has been little alteration in his condition, but he has developed a Dupuytren's contracture in the left hand and continues to suffer from pain and numbness especially during cold weather.

Past history. Nothing relevant.



FIGURE 1. X-ray of left hand showing bone cysts.

Examination. The cartilaginous swellings in his left hand are easily seen. He also has diffuse atrophic changes associated with telangiectasia in the skin over his left shoulder.

Investigations. X-rays of the left hand show multiple chondromata. A left brachial angiogram (1972) showed a basically normal arterial supply to the hand but with multiple feeding vessels to haemangiomatic malformations, relatively stagnant pools of contrast medium, and early filling of the veins suggesting arterio-venous anastomoses. Skin biopsy from the telangiectatic area on his back (1981/76) showed a loose myxoid appearance in the lower dermis but no other abnormality.

Treatment and progress. No treatment is being given. His condition appears to be static.



FIGURE 2. Angiogram showing vascular malformations.

(8) Pemphigus foliaceus associated with S.L.E.

DR B.R.ALLEN AND DR W.G.REEVES

General Hospital, Nottingham

V.G., male, aged 41 years. Hospital case no. 124281.

History. He was first seen in February 1973, with a 3 month history of crusted lesions developing on his trunk. A clinical diagnosis of pemphigus foliaceus was supported by the histology. Nikolsky's sign has occasionally been demonstrated. Immunological studies (see below) were first carried out in January 1976.

In April 1976 he developed tinea corporis which was treated with miconazole. One year later it returned but responded to griseofulvin therapy. In July 1977 he developed severe right sided herpes

zoster in the distribution of T11. In August 1977 he was admitted to hospital because of malaise and weight loss and investigations included a renal biopsy. His pemphigus foliaceus was active again and his prednisolone was raised to 15 mg daily.

Examination. There are no cutaneous signs of S.L.E. but he usually has a few lesions characteristic of pemphigus foliaceus.

Investigations. Haemoglobin, W.B.C., urea and electrolytes, liver function tests were all normal. W.R.—negative. Rose-Waaler—negative.

Immunological investigations. IgA—250 mg/100 ml; IgG—1500; IgE—176 (raised); IgM—110. Indirect test for skin antibodies—persistently negative. Nuclear antibodies positive 1 in 400. IgG class otherwise tissue specific antibodies negative. DNA binding 49% (normal less than 20%) rising to 67%. C3 persistently low at 63–90 mg/100 ml. C4 was normal or slightly reduced. Anti-complementary activity negative.

Skin biopsy (1669/73) showed tiny foci of cleavage beneath the granular layer with just a few acantholytic dyskeratotic cells.

Skin biopsy immunofluorescence normal skin. There is granular staining at the epidermo-dermal junction for IgA, IgM, C3 and C1q. There is also positive even epidermal staining for IgG in an intercellular pattern which extends throughout all layers. These changes are typical for systemic lupus and pemphigus respectively.

Renal biopsy—immunofluorescence. Scattered granules of IgA, IgM and to a lesser extent IgG, C3 and fibrin on the basement membrane.

Comment. Concurrence of pemphigus vulgaris and S.L.E. has been described (Whittingham & MacKay, 1971). Our patient is unusual in that his indirect test for pemphigus antibodies has been persistently negative, against different substrates, and his skin has shown the immunological changes of pemphigus and S.L.E. at the same time. Although he has never had symptoms suggestive of S.L.E. it is possible that these have been masked by the prednisolone he has received for his pemphigus.

REFERENCE

- WHITTINGHAM, S. & MACKAY, I.R. (1971) The 'pemphigus' antibody and immunopathies affecting the thymus. *British Journal of Dermatology*, **84**, 1.

(9) Multiple histiocytomata and systemic lupus erythematosus

DR M. J. CHEESBROUGH FOR DR B.R. ALLEN

General Hospital, Nottingham

E.S., female, aged 49 years. Hospital case no. 111536.

History. She had thrombophlebitis after the birth of her first child and has had leg ulcers for about 19 years on the left leg. She was under the care of Dr Savage in Doncaster from September 1958 until 1968 because of multiple histiocytomata. Of interest at the time was thrombocytopenia with a platelet count of $74,000 \text{ mm}^{-3}$ in 1958 and down to 42,000 in April, 1962. From time to time histiocytomata on the legs have broken down and ulcerated. She was presented to the Section of Dermatology of the Royal Society of Medicine in November 1961. In September 1966 she developed sudden right homonymous hemianopia and on investigation in Sheffield in June 1967, E.E.G., carotid arteriogram and gamma scan were all normal. She had a second undocumented 'stroke' about 1970—she was

not admitted to hospital. She has been attending the Skin Department, Nottingham, continuously for the past 6 years because of leg ulcers which at times are painful and have failed to heal despite out-patient and in-patient dressings.

Past history. Rheumatic fever as a child.

Examination. Vasculitic-looking ulcer of the right lower leg. Variable livedo reticularis. Blood pressure 180/110 mmHg. Heart—mid systolic murmur at the apex. Respiratory system and abdominal system normal. C.N.S.—right homonymous hemianopia. Brisk reflexes. She has slight nasal dysarthria and negative Tensilon test; the neurological signs were felt to be consistent with a pseudobulbar palsy due to a double hemiparesis from past vascular disease.

Investigations. Haemoglobin 13.5 g/100 ml; W.B.C. 12,700/mm³; (neutrophils 10,922); platelets 91,000/mm³; E.S.R. 50 mm/h; urea 10.2 mmol/l; creatinine 130 µmol/l; creatinine clearance 88 ml/min; 24 h urine—protein 1.1 g, Latex R.A. test negative. Rose-Waaler test negative. Autoantibodies: antinuclear factor positive, less than 1 in 100. Thyroid microsome titre 6400. Immunoglobulins: IgA 310; IgG 1150; IgM 88; C3 100 mg/100 ml. C4 25. DNA Antibody 25–30%.

Direct immunofluorescence of light exposed skin showed IgA and IgM in sub-epidermal deposits and fibrin in walls of small blood vessels. There was slight granular deposit of IgM at the D.E. junction and no deposit of complement. Four biopsies of skin lesions confirmed histiocytomata.

Comment. There is evidence of chronic intermittent multisystem disease and a raised DNA binding suggestive of low grade systemic lupus erythematosus but we cannot connect this with her multiple histiocytomata.

(10) Systemic lupus erythematosus with butterfly rash

DR D.M.R.GUNAWARDENA AND DR A.E.D.NAVARATNAM

Derby Royal Infirmary, Derby

L.K., female, 18 years.

History. In July 1976, she was first seen in the Out-Patient Department complaining of an erythematous rash on the face, of 6 months' duration and on the fingers of both hands since the summer of 1975. There was also a history of malaise, lassitude and pain in the left knee joint for 1 year. Her weight remained steady. No relevant drug history.

Family history. No illness of note.

Examination. (August 1976) She was clinically anaemic but afebrile and there was no lymphadenopathy. Diffuse thinning of scalp hair was noted. On her face she had a symmetrical, erythematous, scaly rash on the cheeks, nose, forehead, chin and ears. On the cheeks and nose it had the characteristic butterfly appearance; the areas covered by spectacles were spared (Fig. 1). There were erythematous papules on the palms and dorsal aspects of the fingers on both hands. Other systems were normal.

Investigations. (August 1976) Haemoglobin 9.4 g/100 ml; W.B.C. 5000/mm³; E.S.R. 118 mm/h. Platelets 305 × 10⁹/l; Serum iron 7 µmol/l. Latex smear positive for L.E. cells. Antinuclear factor—positive titre 1/3200 (homogenous pattern). Thyroid cytoplasmic antibodies positive. Serum proteins 87.5 g/l; albumin 38.2 g/l; globulin 49.3 g/l. Electrolytes, blood urea and creatinine clearance normal. Liver function tests, E.C.G. and chest X-ray normal. Direct Coombs' test negative. Rheumatoid factor negative.

Skin biopsy. This showed an atrophic epidermis which lacked rete pegs. There was flaky hyperkeratosis with keratotic plugging of hair follicles and focal liquefaction degeneration of the basal layers

with pigmentary incontinence. The upper dermis below the degenerate epidermis was oedematous. There was a mild patchy inflammatory cell infiltrate in the dermis. The appearances are consistent with systemic lupus erythematosus.

Treatment and course. She was treated systemically with prednisolone 40 mg/day, azathioprine 50 mg t.d.s. and oral iron and topically with Locoid 0.1% cream and Spectraban lotion for the face. By November 1976, her general well-being had improved; (Haemoglobin 13.8 g/100 ml, E.S.R.



FIGURE 1. Symmetrical erythematous scaly rash on face in butterfly distribution.

20 mm/h). The facial rash has not improved and is embarrassing to the patient. A cosmetic cover cream has been prescribed.

Comments. This patient's initial manifestations were cutaneous. In a series of 200 cases (Larson, 1961), 13.5% and in another series of 500 cases (Dubois & Tuffanelli, 1964), 7.6% had initial cutaneous presentations. The facial rash showed little or no improvement with treatment.

REFERENCES

- LARSON, D.L. (1961) *Systemic Lupus Erythematosus*. Churchill Livingstone, London.
 DUBOIS, E.L. & TUFFANELLI, D.L. (1964) Clinical manifestations of systemic lupus erythematosus. *Journal of the American Medical Association*, **190**, 104.

(11) Chronic mucocutaneous candidiasis

DR B.R.ALLEN

General Hospital, Nottingham

H.C., female aged 26 years. Hospital case no. 354230 GH.

History. This girl is one of a family described by Stankler & Bewsher (1972).

At the age of 3 (1954) she developed something resembling a malabsorption syndrome which settled spontaneously in 1 year. A large liver and spleen were noted at that time and 2 years later a liver biopsy was reported as being 'compatible with late effects of hepatitis'. In 1964 (aged 13) she was investigated for failure to thrive and tetany which were attributed to malabsorption. A gluten-free diet failed to help. At the age of 15 (1966) primary hypoparathyroidism and candidiasis were diagnosed and she was put on calcium and ergocalciferol. A year later she was found to have positive adrenal and thyroid antibodies, bilateral perceptive deafness and subcapsular cataracts. In 1970 (aged 19) she had an episode of iridocyclitis and corticosteroids were started because her persistently abnormal liver function tests were attributed to an auto-immune process. They have been continued because of adrenal failure.

Between 1974 and 1977 she was under the care of the Hammersmith Hospital and in October moved to Nottingham and attended the endocrinology clinic (Dr R.B.Tattersall) complaining of infrequent periods, with amenorrhoea since July, sensitivity to the cold and hair loss. Investigations showed poor thyroid and ovarian function and she was started on thyroxine. Her hair loss was very extensive and the presence of exclamation mark hairs left little doubt that she was suffering from alopecia areata.

Family history. The clinical manifestations in the patient, her affected brother and non-identical twin sister are given in the following table.

	Sister	H.C.	Brother
Ungual candidiasis	+	+	+
Oral candidiasis	+	+	+
Alopecia areata	+	+	+
Hypoparathyroidism	+	+	+
Thyroid deficiency	o	+	o
Adrenal deficiency	o	+	o
Ovarian deficiency	o	+	o

Mother, father and one brother are normal.

Examination. She is of small stature and has telangiectasia of the cheeks which in the past has been attributed to steroid therapy. Oral and ungual candidiasis is evident and she has extensive alopecia areata.

Investigations. Serum thyroxine 128 nmol/l (50-140); T₃ uptake 110 (118-90); Free thyroxine index 116 (48-134); T.S.H. 9.0 mu/l (normal up to 6 mu/l); F.S.H. 95 u/l (4.4-14.3), L.H. 43 u/l (1.7-12.9).

Immunological investigations. These will be presented in detail at the meeting.

Treatment. Thyroxine 100 µg daily, calciferol 50,000 u alternate days, calcium Sandoz 2 tablets daily, hydrocortisone 20 mg daily, fludrocortisone 0.025 mg alternate days, Betnesol eye drops, ferrous fumerate 200 mg b.d.

Discussion. At the time of the report by Stankler & Bewsher, Mrs H.C. and her brother had not suffered from alopecia areata although her non-identical twin sister had.

REFERENCE

- STANKLER, L. & BEWSHER, P.D. (1972) Chronic mucocutaneous candidiasis, endocrine deficiency and alopecia areata. *British Journal of Dermatology*, **86**, 238.

(12) R.E.M. syndrome

DR B.R.ALLEN

General Hospital, Nottingham

I.T., female, aged 22 years. Hospital case no. 135601.

History. This lady was first seen in 1976 with a 3 year history of an erythematous, maculo-papular eruption on her upper chest and back. This was improved by sunlight and made more obvious by heat. Irritation was only slight. She had received no medication for it and the only drug she has taken regularly is the contraceptive pill (Eugynon 30).

Past illnesses. No previous skin trouble.

Family history. One brother has eczema.

Examination. A maculo-papular, non-scaly eruption on the upper chest and central back.

Investigations. Skin biopsy: 'The epidermis was thin with a narrow sub-epidermal zone of oedematous homogenization of the dermis. The upper dermal capillaries show mild lymphocytic cuffing. Multiple Alcian-blue positive foci can be stained throughout the dermal connective tissue.'

Progress and treatment. Local treatment with topical steroids and systemic metronidazole and oxytetracycline have been of no benefit, but there has been spontaneous improvement of her back.

Comment. Clinical and histological features are consistent with the so-called R.E.M. syndrome described by Steigleder *et al.* (1974).

REFERENCE

- STEIGLEDER, G.K., GARTMANN, H. & LINKER, U. (1974) R.E.M. syndrome: reticular erythematous mucinosis (round cell erythematosis), a new entity? *British Journal of Dermatology*, **91**, 191.

(13) Familial angio-oedema—C1 esterase inhibitor deficiency

DR M.J.CHEESBROUGH FOR DR P.D.C.KINMONT

General Hospital, Nottingham

P.C., female, aged 22 years. Hospital case no. 332736 GH.

History. She has had swellings usually of the right hand and/or both knees for about 11 years—on average about once a month. The face has been affected once but never the mucous membranes.

Episodes usually last a couple of days and are not precipitated by any known factor. There is no history of abdominal pains.

Family history. Her father and one brother of six siblings are known to be affected.

Examination. She is normal between attacks but when seen on one occasion she had soft tissue swelling of the right hand and forearm.

Investigations. C3 126 mg/100 ml; C4 6 mg/100 ml; C1 esterase inhibitor 13% of normal; IgA 110 mg/100 ml (normal 125-425); IgG 1000 mg/100 ml (normal 500-1600); IgM 125 mg/100 ml (normal 50-175); IgE 165.

Treatment. Tranexamic acid 500 mg three times a day since August 1977.

Comment. There is no evidence at this stage that the tranexamic acid is helping her.

(14) Angio-oedema—? acquired C1 esterase inhibitor deficiency

DR M.J.CHEESBROUGH FOR DR P.D.C.KINMONT

General Hospital, Nottingham

G.F. female, aged 62 years. Hospital case no. 100069.

History. Since July, 1976, she has had about six episodes lasting 3-5 days of swelling of the face, including the eyes and lips, difficulty in swallowing and slight tightness on breathing. One attack was followed by abdominal pain and diarrhoea. Between attacks she has had several episodes of upper abdominal pain lasting about 1 h, following which she passes a small quantity of mucus rectally. Her general health is good.

Family history. None of her relatives are, or have been, known to be affected.

Investigations. C3 169 mg/100 ml; C4 not detected, C1 esterase inhibitor 34% of normal; IgA 500 mg/100 ml (normal 125-425); IgG 930 mg/100 ml (normal 500-1600); IgM 180 mg/100 ml (normal 50-175); IgE 155 mg/100 ml. Electrophoresis—monoclonal IgA band is seen migrating towards the anode, appearing in the same region as C1 esterase migrates. Anti-C1 esterase antibody precipitates the band. The following are normal—barium meal and follow through, barium enema, chest X-ray, liver scan, liver function tests, blood count, auto-antibodies, cryoglobulins, cold agglutinins.

Treatment. Tranexamic acid tablets 500 mg three times a day.

Comment. There is a possibility that the 'benign' band of monoclonal IgA is inactivating the C1 esterase inhibitor. An underlying lymphoma has been considered but no signs have yet been found.

(15) Acquired C1 esterase deficiency with cold agglutinin disease associated with monoclonal IgM antibodies

DR T.B.WALLINGTON FOR DR E.A.FRENCH AND DR W.G.REEVES

University and General Hospitals, Nottingham

A.G., male, aged 76 years. Hospital case no. 339476.

History. His first complaint was of Raynaud's phenomenon affecting his hands, feet and face for approximately 2 years. He also described darkening of his urine 2 h after cold exposure sufficient to

produce Raynaud's phenomenon. This soon resolved but was accompanied by mild abdominal pain. During this period he had also suffered a mild attack of herpes zoster on the right side of his chest which cleared within 4 weeks. No family history of angio-oedema.

Examination. No abnormality discovered: in particular no evidence of hepatosplenomegaly or lymphadenopathy. His toes and feet became cold and blue during the examination.

Investigations. Haemoglobin 10.5 g/100 ml; leukocytes $10.0 \times 10^9/l$ with normal differential. Reticulocytes 3.6%. No abnormal cells were seen in peripheral blood or bone marrow aspirate. Immuno-haematological studies showed the presence of a cold-agglutinin which agglutinated pooled adult blood group O cells but not cord blood cells and therefore probably has I specificity. Bilirubin was raised at 86 $\mu\text{mol/l}$. Low titre anti-platelet antibodies were also detected. Radiological examination including lymphangiography was normal.

Immunological investigations. Serum immunoglobulin levels were normal apart from a raised IgM level of 800 mg/100 ml. Immuno-electrophoresis showed the presence of monoclonal IgM (Kappa). Free light chains were not detected in concentrated urine. Complement estimation revealed undetectable C4, C3 of 96 mg/100 ml and CH50 titre of less than 10. Significant anti-complementary activity was detected. The C1 esterase inhibitor level was 12% normal. Cryoglobulins were not detected and no other auto-antibodies or rheumatoid factor was detected. Peripheral blood T and B lymphocytes were present in normal numbers.

Comment. This patient shows the interesting combination of C1 esterase inhibitor deficiency, cold agglutinin disease and an IgM Kappa paraprotein. It is clear from his personal and family history that the C1 esterase inhibitor deficiency is acquired rather than inherited. It is also asymptomatic. These abnormalities have been described in association with lymphoma. In some instances the low C1 esterase inhibitor level is accounted for by the sequestration of the enzyme by binding to abnormal cells. It is also possible that the inhibitor could be bound to the abnormal antibody and although reduced on immuno-chemical testing could still be present in a functionally active form.

REFERENCES

- CALDWELL, J.R., RUDDY, S., SCHUR, P.H. & AUSTEN, K.F. Acquired C1 esterase inhibitor deficiency in lymphosarcoma. *Clinical Immunology and Immunopathology*, **1**, 39.
 SCHREIBER, A.P., WEIMAN, B., ATKINS, P., GOLDWEIN, F. & PIETRA, G. (1976) Acquired angio-oedema with lymphoproliferative disorder. *Blood*, **48**, 567.

(16) Spasticity and vascular lesions

DR B.R.ALLEN

General Hospital, Nottingham

A.K., male, aged 10 years. Hospital case no. 224908.

History. He was born at the City Hospital, Nottingham, a full term baby weighing 5 lb 4 oz. His progress was uneventful until the age of 2 when he developed chilblain-like lesions on his hands, feet and ears which lasted the whole winter. He has never been free of lesions since that time, but there is quite definite deterioration during the colder months. Shortly after the skin lesions developed he started to have difficulty walking and spasticity of the lower limbs was noted. This has not been progressive. In November 1974 he was referred from the Paediatric Assessment Unit (Dr D.H.Mellor) for an opinion on his skin lesions.

Family history. His younger brother (Case no. 17) is similarly affected. Both parents are normal and there is no consanguinity.

Examination. He is a mentally normal boy with spasticity of both lower limbs. There are scars and cyanotic lesions on his toes, hands (both palmar and dorsal aspects), knees, elbows and ears. He has also developed plaques of erythema around his eyes on occasions. Two telangiectatic areas have been seen on his feet reminiscent of early angiokeratomata of Mibelli. Hyperkeratosis has been absent.

Investigations. Skin biopsy 3009/75 (of a fresh lesion less than 24 h old) 'shows oedema of the papillary part of the dermis, with accentuation of the rete pegs and slight parakeratosis. The dermal capillaries are prominent and there is heavy perivascular infiltration with lymphocytes, some histiocytes and occasional neutrophils, the latter mainly in the superficial zone. The appearances would do quite well for pernio (chilblain)' (Dr G.F.M. Hall).

Immunofluorescent examination showed a speckled deposition of C₃ at the dermal-epidermal junction and also the suggestion of a minor degree of deposition of IgA in a similar situation. Lym-



FIGURE 1. Vascular lesion on left foot.

phocytic irradiation studies (Dr A.M.R.Taylor, Department of Cancer Studies, Birmingham)—‘The aberration frequency in irradiated lymphocytes (400 rads, 300 KeV X-rays) is normal.’ Extensive metabolic and genetic studies have failed to show anything abnormal.

Treatment. In October, 1976, dapsone was started in a dose of 25 mg twice daily because of the similarity between some of the lesions and erythema elevatum diutinum. The skin appeared to improve but dapsone had to be discontinued because of haemolysis and methaemoglobinaemia. This was followed by a rapid deterioration of his skin lesions.

(17) Spasticity and vascular lesions

DR B.R.ALLEN

General Hospital, Nottingham

C.K., male, aged 8 years. Hospital case no. 133126.

History. He was a normal full term baby who weighed only 4lb 12oz. At birth he was pale and covered with meconium but did well for 2 weeks after which he became irritable, lethargic and pyrexial. Investigations for a viral encephalitis were negative. The difficulties persisted for the first year of life and at 13 months a severe respiratory infection was followed by spastic quadriplegia and epilepsy. Chilblain-like lesions developed at the age of 18 months. He also has congenital glaucoma of the left eye.



FIGURE 2.

Examination. A grossly retarded boy with lesions similar to his brother's.

Investigations. As with his brother, extensive investigations have failed to reveal any abnormality.

Treatment. Between December 1976 and April 1977 he received dapsone 10 mg t.d.s. with apparent benefit.

(18) Congenital hypotrichosis—for diagnosis

DR M.J.CHEESBROUGH FOR DR P.D.C.KINMONT

Children's Hospital, Nottingham

I.M., male, aged 4 years. Hospital case no. 146131 (Children's).

History. He was born without scalp hair, eyelashes or nails and since then his hair has grown slowly and his nails normally. He sweats normally. There is no family history and no consanguinity. His growth and development have been normal. He had two febrile convulsions at the age of 12 and 15 months.

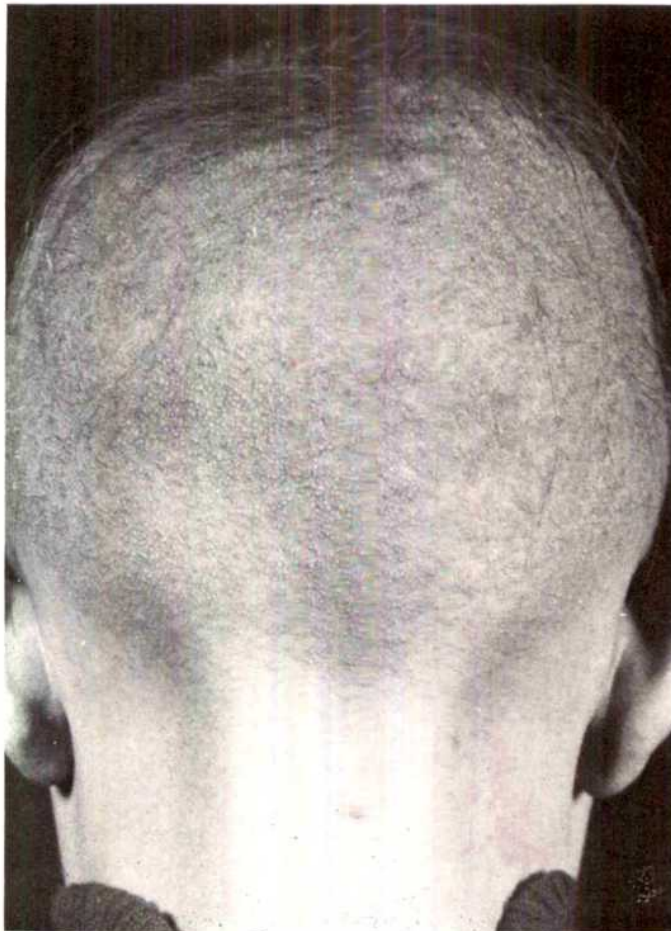


FIGURE 1.

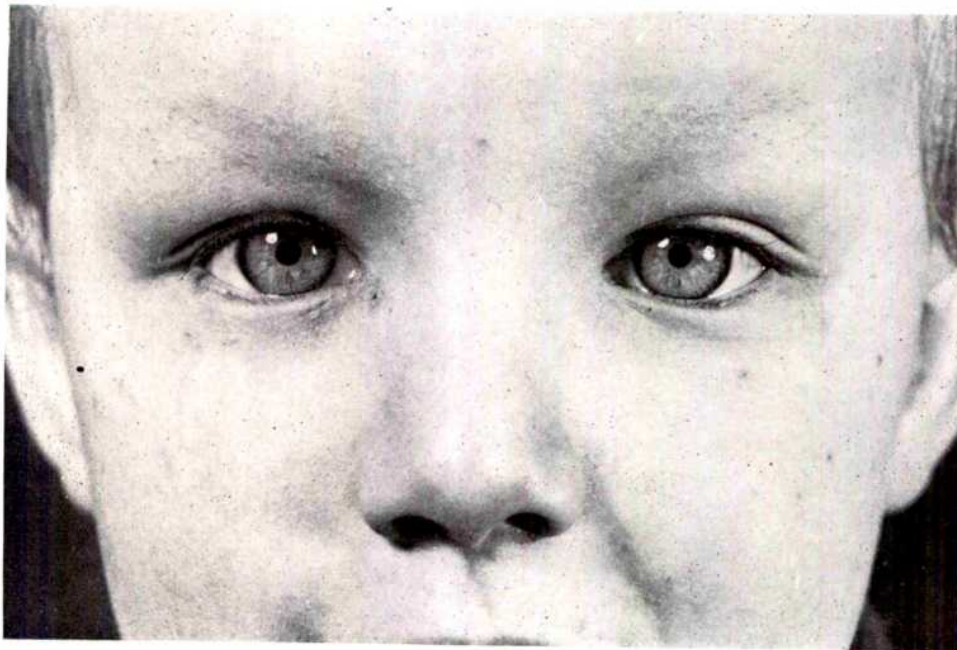


FIGURE 2. Absent eyebrows.

Examination. Scalp hair is sparse, particularly at the sides which show keratosis pilaris. The upper eyelids are sparse and lower lids and eyebrows are absent. The rest of the skin and systemic examination are normal.

Investigations. Urine amino acids normal.

Histology. 'The skin shows an apparently normal number of hair follicles, the epithelium of many appears to be normal but many contain eosinophilic poorly formed hair structures, apparently normal hair roots are seen, sebaceous glands are few and there is a perifollicular fibrosis with mild lymphocytic infiltration' (1761/78) (Dr A.M. MacKay).

Comment. Most reports of congenital hypotrichosis, of whatever type, are of families with several affected individuals. Inheritance is autosomal dominant. This patient is a sporadic case; is he of the Marie-Unna type?

REFERENCE

MULLER, S. (1973) Alopecia: syndromes of genetic significance. *Journal of Investigative Dermatology*, **60**, 475.

(19) Psoriasis with terminal phalangeal osteolysis

DR M.J. CHEESBROUGH FOR DR P.D.C. KINMONT

General Hospital, Nottingham

T.B., male, aged 30 years. Hospital case no. 362491.

History. He has had psoriasis for 12 years, widespread for most of the time. He has been treated topically, with tar and steroid preparations. For a few months he has noticed enlargement of the ends of his fingers and toes, pain under the nails and stiffness of the toes and fingers. The pains have required analgesia.



FIGURE 1. X-ray of hands showing normal joints and osteolysis of the terminal phalanges.

Examination. Widespread psoriasis. There is pitting and onycholysis of all the finger nails with splaying of the distal segments. There are no joint changes.

Investigations. Serum uric acid $470 \mu\text{mol/l}$ (normal $1-400$); X-rays of hands and feet show atrophy of the tufts of the terminal phalanges with soft tissue swelling of the distal segments. No joint erosions are seen.

Comment. Terminal phalangeal osteolysis is one of the radiological signs of psoriatic arthropathy (Avila *et al.*, 1960); it normally, however, occurs in conjunction with frank arthritis.

REFERENCE

- AVILA, R., PUGH, D.G., SLOCUMB, C.H. & WINKELMANN, R.K. (1960) Psoriatic arthritis, a roentgenologic study. *Radiology*, **75**, 691.

(20) Vinyl chloride disease

DR E.N. MITCHELL JOHNSTON

General Hospital, Nottingham

A.D., male aged 40 years. Hospital case no. 125864.

History. He worked as a plasterer until 1969, when he joined a new P.V.C. production plant at Chesterfield. After 3 weeks as an autoclave hand-cleaner he became an A operator controlling the

flow of reactor chemicals and was exposed frequently to vinyl chloride leaking from valves. From 1971 to 1973 on PVC Plastisol production he again had to enter reactors to chip off solid PVC from the walls, wearing gloves and overalls but no respirator. On occasions he became light headed.

During this period Raynaud's phenomenon, stiffness, numbness and burning pain affected his hands (as a plasterer, cold had been normally tolerated). There was increasing thickening of the skin of the upper limbs, trunk and face, and impairment of grip, fine finger movements, trunk rotation and opening of the mouth. Joint pains, loss of energy and weakness of the upper limbs caused him to cease work completely in April, 1973.

There was no history of digital ulceration, dysphagia, tinnitus or change of libido, no history of drugs (smoking and alcohol ingestion were minimal), and no significant history of vibration tool operation.

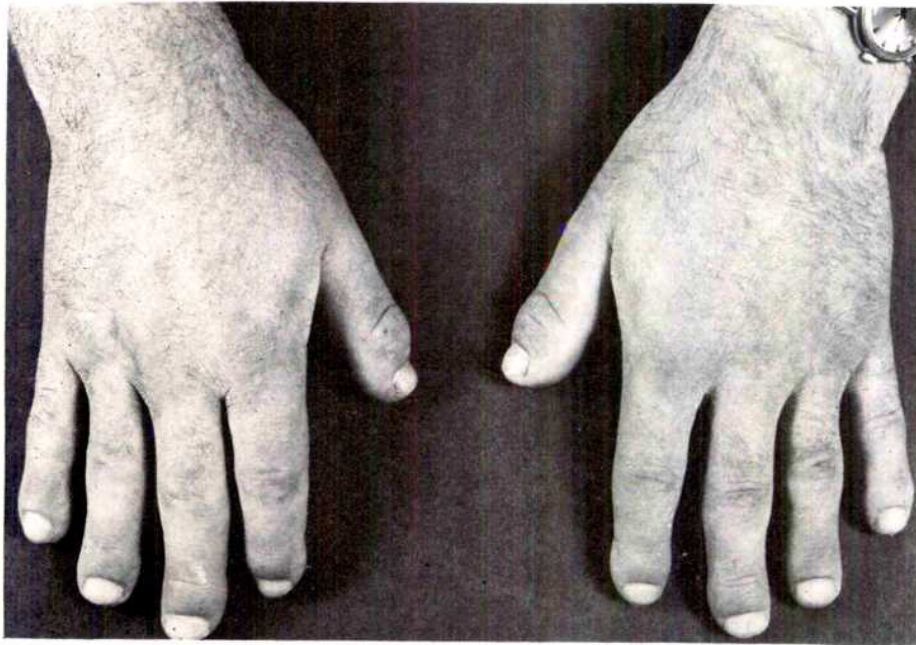


FIGURE 1. Hands showing shortening of the distal segments of fingers.

Family history. Father—severe arthritis and leg thrombosis; mother—asthma since her twenties; brother—migraine.

Examination. (May 1973) Hands were mottled with areas of normal pink, cyanosis and pallor, the latter increasing with finger flexion. All digits except minimi showed convexly curved nails over bulbous, shortened, tender terminal segments, the distal portion of which could be moved in relation to the proximal. The distal interphalangeal joints appeared swollen. No nail fold or macular angiectases, no epidermal atrophy, no trophic lesions, no loss of body hair. The skin of the whole of the upper limbs and of the face, trunk and thighs appeared thickened. This sclerosis made venepuncture very difficult

(he was formerly a blood donor). In several areas, especially around the axillae, the surface of the skin was irregular with papular or cord-like or plaque thickening—the latter being present over the volar aspect of each wrist. The facial features appeared coarsened in contrast to those in old photographs. No other abnormal physical signs.

Investigations. Haemoglobin, white cell count, electrolytes, serum calcium and phosphate, liver and thyroid function tests, Rose-Waaler, urine including calcium and hydroxy-proline estimations, and E.C.G. were normal. E.S.R. 35 mm/h. Plasma viscosity slightly elevated. Platelets (6/64) 190,000/mm³; (1/75) 115,000/mm³. Isotope scan (1975) liver normal; spleen definitely enlarged.

Histology. Professor J.A.Milne commented on the biopsy of finger skin in 1973 'Thickening and separation of collagen fibres is as described in Occupational A.O.L. by Markowitz but no Alcian blue nor toluidine blue positive material seen between collagen fibres, and elastic tissue pattern is within normal limits in this section'.

Immunological investigations. Serum auto-antibody screen negative, except for low titre antinuclear antibody. Skin biopsy, May 1974—Increased staining is seen with IgG throughout the dermis. A little increased staining is seen with IgA but an entirely normal pattern is seen with IgM, C₃ and fibrinogen reagents. Dr A.M.Ward commented: 'A skin biopsy taken at the time of exploration of his mandibular ramus showed an immune complex vasculitis with deposition of C₃ and IgG in the media of small and medium sized arterioles. The immuno-globulins at the time were not normal, with an IgG of 19 g/l. IgA and IgM normal. No cryoglobulins and no cryofibrinogen have been detected. The polyclonal increase in IgG and an immune vasculitis are common to all the patients with V.C. disease and are usually associated with an immune complex disorder with *in vivo* complement consumption via the classic activation pathway.'

Radiological investigation. Hand (May 1973)—The abnormalities included dissolution of the central parts of all the terminal phalanges except minimi, erosion of all the terminal tufts and thinning of the central part of middle phalanges.

Mandible—Considerable erosion of both ascending rami causing marked thinning of the bone and on the left side this has extended upwards to involve the condyle.

Erosions were also seen in the following bones, on one or both sides: first metacarpal, ulna, radius, humerus, clavicle, pelvis, femur, patella, tibia, fibula, calcaneum and first metatarsal. The following have been normal: chest, skull and spine, barium swallow and meal (5/1973 and 10/1975).

Progress films 6 months later showed a central defect in the distal phalanx of the little finger (which was now seen clinically to be shortened) and further osteolysis of the distal phalanges of the fingers and great toes. At 2 years some erosions were slightly larger.

Arteriography of the hands (11/74) showed persistent occlusions or narrowings of some digital arteries. Hypervascularity was seen in the terminal portions of the fingers.

Lung function tests. Dr C.S.Darke commented: 'Perfusion scan showed uneven appearance throughout both lungs, but particularly at the right base. Total respiratory resistance was increased at 5 cm of water per litre indicating some restrictive abnormality. There was also some delay in gas transfer.'

Progress. During the past 5 years there has been a gradual reduction in the thickness of the skin as judged clinically. In 1975 attacks of central abdominal pain occurred but these have become less frequent.

Comments. At the P.V.C. factory a number of other workers developed various abnormalities including two with Raynaud's phenomenon, but this patient was the only one to show scleroderma-like changes and bone lesions. The latter are far more extensive than in most recorded cases. Meyerson & Meier (1972) reported a non-occupational case of acro-osteolysis, erosions of the mandibular condyles and 'unique cutaneous lesions'. Markowitz *et al.*, (1972) and Harris & Adams (1967) both described plaque-like thickening of the skin at the wrists.

REFERENCES

- MARKOWITZ, S.S., McDONALD, C.J., FETHIERE, W. & KERZNER, M.S. (1972) Occupational acro-osteolysis. *Archives of Dermatology*, **106**, 219.
- MEYERSON, L.B. & MEIER, G.C. (1972) Cutaneous lesions in acro-osteolysis. *Archives of Dermatology*, **106**, 224.
- KEWIN HARRIS, D. & ADAMS, W.G.F. (1967) Acro-osteolysis occurring in men engaged in polymerization of vinyl chloride. *British Medical Journal*, **iii**, 712.

(21) Partial lipodystrophy

DR B.R.ALLEN

General Hospital, Nottingham

C.M. female, aged 58 years, Hospital case no. 362275.

History. This lady was referred in October 1977 by the general physicians who were investigating her for suspected temporal arteritis. Lack of fat on the upper half of her body was noticed. This was



FIGURE 1. Facial appearance.

lost suddenly when she was about 13, and caused her great embarrassment. As a result, when she was in her twenties, an attempt was made to transplant fat from her abdominal wall into her cheeks. The transplanted fat became involved in the same process.

Examination. She has the typical facies of lipodystrophy. One small pad of the fat remains on her left cheek.

Investigations. A skin biopsy from her forearm showed few changes other than a relative loss of fat. Direct immunofluorescence was reported as showing 'no abnormal immunoglobulin or complement deposits of diagnostic significance'. Temporal artery biopsy normal; haemoglobin 14.5 g/100 ml; E.S.R. 6 mm/h; W.B.C. 7400/mm³; creatinine 74 μ mol/l; urea and electrolytes normal; urine—no proteinuria; serum complement levels normal C3 and C4, autoantibody screen negative.

Treatment. Nil apart from Cologel for diverticulitis.

Comment. In the absence of clinical evidence of renal involvement, a biopsy was not felt to be indicated.

(22) Partial lipodystrophy and glomerulonephritis

DR R.P. BURDEN

City Hospital, Nottingham

M.W., female, aged 28 years.

History. This patient was first recognized as having partial lipodystrophy at the age of 25 when she presented with a 2 month history of ankle swelling and was found to have proteinuria. As far as she was aware her face had always been thin and there had been no recent change in her appearance.

Examination. Loss of subcutaneous fat particularly in the cheeks but also, to a lesser extent, over the shoulders. She was normotensive and, apart from ankle oedema, there were no other abnormal physical signs.

Investigations. Nephrotic syndrome was confirmed with hypoproteinaemia (25 g/l) and heavy proteinuria (13.8 g/24 h) which was non-selective. Serum creatinine 95 μ mol/l; A.S.O. titre 100 units/ml; W.R. and antinuclear antibody negative. The C3 component of complement was reduced at 13 mg/100 ml (normal 80–120) but C4 was normal at 40 mg/100 ml. C3 nephritic factor was present.

Renal histology: Light microscopy. There was slight hypercellularity in all glomeruli with marked increase in mesangial substance which in places extended into the peripheral capillary loops. Methenamine silver stains showed irregular reduplication of the basement membrane. Tubules, vessels and interstitial tissues were normal.

Electron microscopy confirmed the above abnormalities and demonstrated extensive uniform thickening of the basement membranes with both intramembranous and subendothelial dense deposits.

Immunofluorescence. There were heavy deposits of C3 and, to a much lesser extent, IgM along the basement membrane.

Skin histology. A specimen of skin from the forearm was normal on light microscopy and on immunofluorescence.

Discussion. Since the association between partial lipodystrophy (PLD) and renal disease was documented in 1958 (Gellis *et al.*, 1958) further studies have revealed an immunological basis for the renal complication (Peters *et al.*, 1973; Sissons *et al.*, 1976). The renal histological appearances in this

patient (and in others with PLD) were those of membrano-proliferative glomerulonephritis (MPGN). This form of glomerulonephritis occurs more commonly in the absence of PLD and is associated with low concentrations of C3 complement and with the presence of C3 nephritic factor, capable of activating C3. Both hypocomplementaemia and C3 nephritic factor are associated with PLD and may antedate the nephritis (Thompson & White, 1973). This suggests that in patients with MPGN, with or without PLD, complement abnormalities predispose to the development of immune complex mediated nephritis perhaps as a result of recurrent chronic infections. However, heavy immunoglobulin deposits are rarely seen in the glomeruli in MPGN contrasting with the heavy basement membrane deposits of C3 and suggesting that this may not be an example of immune complex disease. An

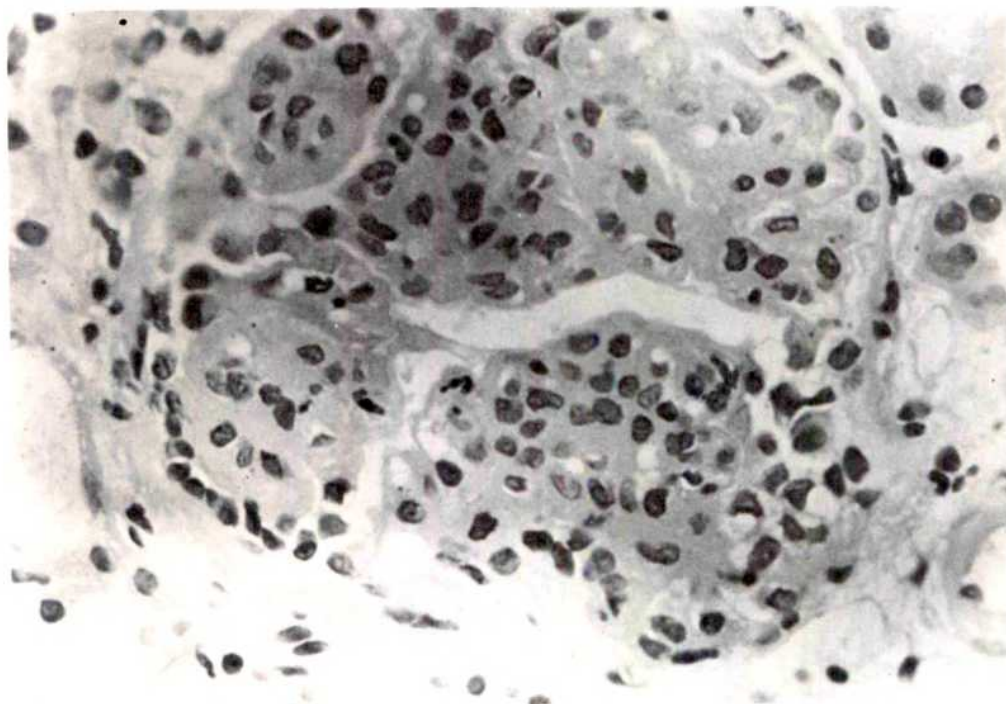


FIGURE 1. Solitary glomerulus showing hypercellularity and marked increase in mesangial substance.

alternative explanation is that C3 activation alone may lead to the glomerular injury (Sissons *et al.*, 1976).

In MPGN without PLD persistent hypocomplementaemia is associated with the presence of intramembranous rather than subendothelial dense deposits, whereas in patients with PLD the deposits are characteristically intramembranous (Habib *et al.*, 1975). In this respect the present patient is unusual although subendothelial deposits have been recorded in at least one patient of a previous series (Sissons *et al.*, 1976).

The natural history of the renal abnormalities associated with partial lipodystrophy is one of progressive renal failure but as yet there has been no evidence of impaired renal function in this patient during the 18 months she has been under observation. In spite of the advances that have been

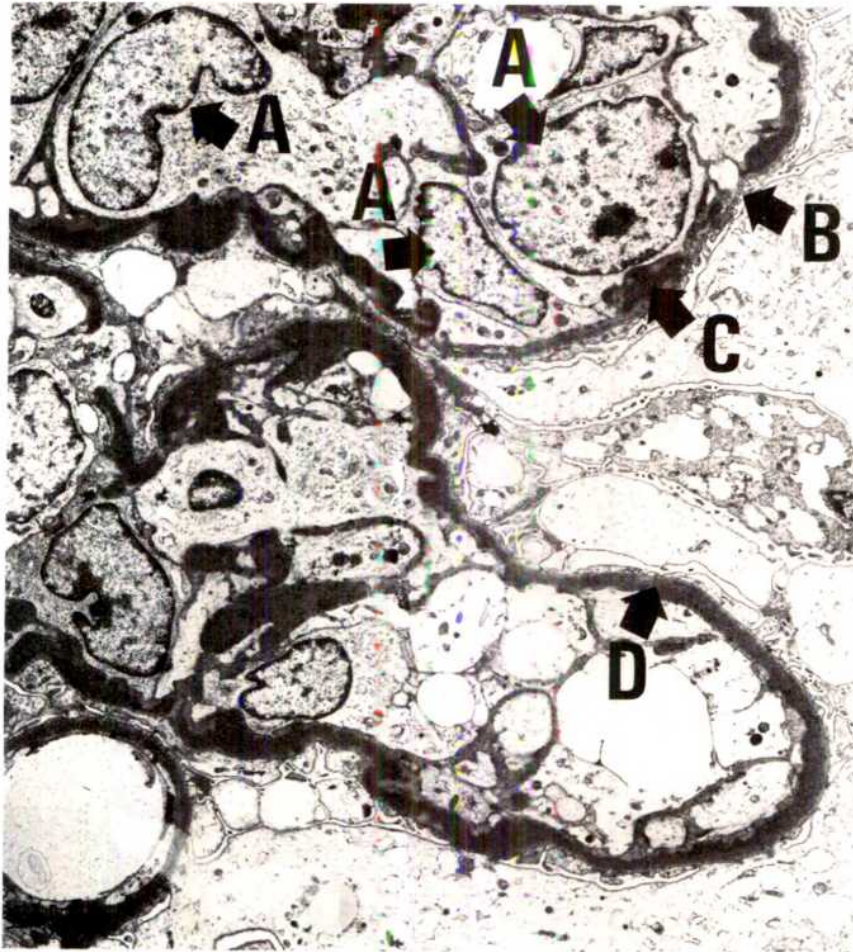


FIGURE 2. Electron micrograph of part of a glomerulus showing A endothelial hypercellularity and B glomerular basement membrane with C subendothelial and D intramembranous dense deposits.

made regarding the pathogenesis of the renal lesions, the cause of the lipodystrophy and the associated complement abnormalities remain obscure.

Acknowledgments. I thank Professor R.E.Cotton for the renal histology, Dr W.G.Reeves for the immunological studies and Dr A.Stephens for the electron micrograph.

REFERENCES

- GELLIS, S.S., GREEN, S. & WALKER, D. (1958) Chronic renal disease in children with lipodystrophy. *American Journal of Diseases in Childhood*, **96**, 605.
- HABIB, R., GUBLER, M.C., LOIRAT, C., MAÏZ, H.B. & LEVY, M. (1975) A variant of membranoproliferative glomerulonephritis. *Kidney International*, **7**, 204.
- PETERS, D.K., WILLIAMS, D.G., CHARLESWORTH, J.A., BOULTON-JONES, J.M., SISSONS, D.G.P., EVANS, D.J., KOURILSKY, O. & MOREL-MARDGER, L. (1973) Mesangiocapillary nephritis, partial lipodystrophy and hypocomplementaemia. *Lancet*, **ii**, 535.
- SISSONS, J.G.P., WEST, R.J., FALLOWS, J., WILLIAMS, D.G., BOUCHER, B.S., AMOS, N. & PETERS, D.K. (1976) The complement abnormalities of lipodystrophy. *New England Journal of Medicine*, **294**, 461.
- THOMPSON, R.A. & WHITE, R.H.R. (1973) Partial lipodystrophy and hypercomplementaemic nephritis. *Lancet*, **ii**, 697.

(23) Dermatitis herpetiformis

DR E.D.A. MITCHELL FOR DR B.R. ALLEN AND DR W.G. REEVES

General Hospital, Nottingham

L.M., female, aged 61 years. Hospital case no. 327887.

History. In November 1976, she suddenly developed widespread annular areas of erythema and blistering on her trunk and limbs. The blisters were up to 2 cm in diameter and the irritation was severe.

Past history. Chronic bronchitis for 10 years.

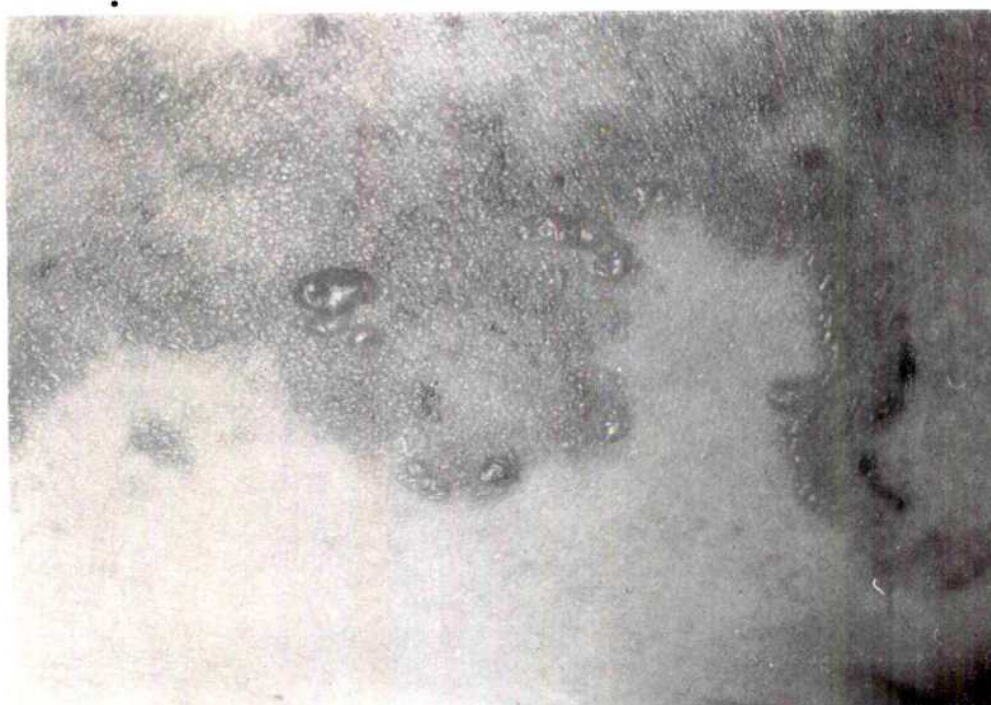


FIGURE 1. Trunk showing erythema and blisters

Investigations. Haemoglobin 13.8 g/100 ml; W.B.C. 9000/mm³; E.S.R. 28 mm/h. Liver function tests and urea and electrolytes normal. Chest X-ray normal. Immunoglobulins: IgA 310 (normal 125-425); IgG 820 (normal 500-1600); IgM 58 (normal 50-175); IgE less than 30. Autoantibody screen showed positive smooth muscle antibody in a titre of less than 100. Immunofluorescence of the intact bulla showed bright linear deposition of IgA at the dermal-epidermal junction. There was no staining for other immunoglobulins or complement. Mucosal biopsy normal. A repeat biopsy of clinically uninvolved skin examined by immunofluorescence showed no change from the previous biopsy.

Treatment and course. Clinically the appearances were those of pemphigoid and she was started on prednisolone 40 mg daily. This did not control the eruption and the dose was increased to 80 mg

with only a moderate response. In view of the IgA deposition it was decided to try a course of dapsone. This resulted in a dramatic improvement. She is now on 5 mg of prednisolone daily which she has been on for years for her chest condition. On one occasion the dapsone was stopped with the development of a mild recurrence only after a 6 week interval.

Comment. This lady is one of a small number of patients with dermatitis herpetiformis in whom the deposition of IgA is in a linear fashion at the dermo-epidermal junction. Whether such patients are a distinct sub-group is not clear. Chorzelski *et al.* (1971) noted that in two patients with linear IgA deposition the clinical picture mimicked pemphigoid in the acute phase. During remission staining reverted to the more usual granular deposition in the dermal papillae. Our patient has continued to have linear IgA at the dermo-epidermal junction during her remission.

REFERENCE

- CHORZELSKI, T.P., BEUTNER, E.H., JABLONSKA, S., BŁASZCZYK, M. & TRYTSHAUSER, C. (1971) Immunofluorescence studies in the diagnosis of dermatitis herpetiformis and its differentiation from bullous pemphigoid. *Journal of Investigative Dermatology*, **56**, 373.

(24) Epidermolysis bullosa acquisita and Crohn's disease

DR M.J. CHEESBROUGH FOR DR P.D.C. KINMONT

General Hospital, Nottingham

D.N., female, aged 32 years. Hospital case no. 128286.

History. This lady has had psoriasis for about 11 years easily controlled with topical applications. Crohn's disease was diagnosed in 1968 and she had a right hemicolectomy in 1969. Because of relapse she had a proctocolectomy in 1971. Her ileostomy has functioned well since. About 6 months after the proctocolectomy she noticed blistering and ulceration of the hands, elbows and knees after minor knocks. There has never been photosensitivity. There is no family history of psoriasis or any other skin disorder and she takes no drugs.

Examination. At times there have been small blisters and milia on the backs of the hands and elbows. Small scars are evident. Psoriasis is minimal.

Investigations. Examination of urine, ileostomy fluid and blood has been repeatedly negative for excess porphyrins (Nottingham, and University Department of Materia Medica, Stobhill Hospital, Glasgow). Serum zinc 100 $\mu\text{g}/100\text{ ml}$ (normal 60–100); copper 140 $\mu\text{g}/100\text{ ml}$ (normal 80–140). These metals were analysed by atomic absorption by Dr Molokhia in Manchester, 1977. A skin biopsy showed a sub-epidermal blister with mild chronic inflammation of the underlying dermis. Direct immunofluorescence showed linear continuous deposition of IgG at the dermo-epidermal junction and extending into the dermal papillae. Discrete globular IgM is seen at the lower epidermis and some traces resembling cytol bodies. C3 is found in a coarse and irregular pattern mostly above the dermo-epidermal junction and especially near vesicles. Fibrinogen was strongly positive in and around vesicles and continued along the region of the junction adjacent to them. The following were normal: blood count, liver function tests, serum iron, iron binding capacity; thyroid (cytoplasmic and colloid), parietal cell, antinuclear and mitochondrial antibodies—negative; smooth muscle antibody was positive at a titre of 1 in 20.

Treatment. She has been treated with vitamin A 400 mg twice a day for 3 months and zinc sulphate 220 mg twice a day to no effect.

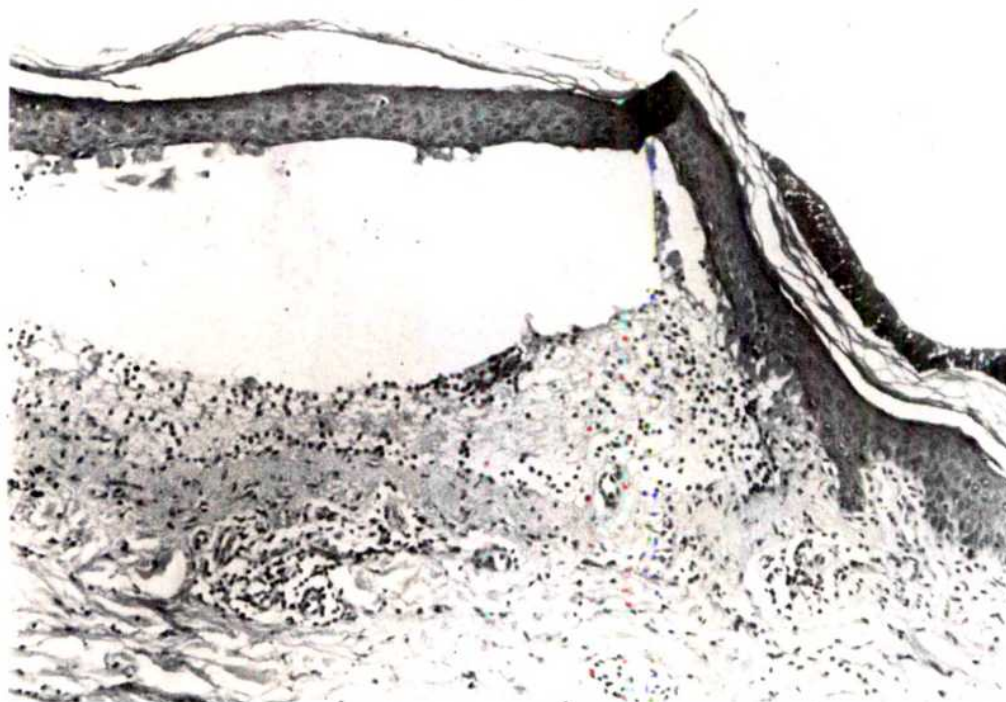


FIGURE 1. Subepidermal blister.

Comment. With the exception of nail dystrophy she fulfils Roenigk's four criteria for E.B.A. Crohn's disease is one of the systemic disorders known to be associated with E.B.A. Our patient's bullous disorder has not coincided with activity of her Crohn's disease.

REFERENCES

- PEGUM, J.S. & WRIGHT, J.T. (1973) Epidermolysis bullosa acquisita and Crohn's disease. *Proceedings of the Royal Society of Medicine*, **66**, 234.
 ROENIGK, H.H., RYAN, J.G. & BERGFELD, W.F. (1971) Epidermolysis bullosa acquisita. Report of three cases and review of all published cases. *Archives of Dermatology*, **103**, 1.

(25) Sarcoid of nose with collapse of nasal cartilage

DR B.R.ALLEN

General Hospital, Nottingham

A.M., female, aged 65 years. Hospital case no. 137106.

History. In 1975 she attended Dr D.Davies (Chest Physician) with a 6 month history of wheezing, dyspnoea, weight loss and lack of energy. Respiratory function tests showed a gross reduction in vital capacity at 1.05 l and F.E.V.1. at 0.55 l. She also complained that for 1 year she had

suffered redness and increasing deformity of her nose, but had not sought advice about it. In January 1976, she was seen in the E.N.T. Department (Mr Neil) for a biopsy to be taken from her palate, which was thickened and ulcerated, and an enlarged supra-clavicular lymph node. The histology was reported as consistent with tuberculosis although no acid fast bacilli were seen, and antituberculous therapy was started. Three months later there was no improvement and the diagnosis of tuberculosis was questioned. A tuberculin test was negative and a Kveim test strongly positive and a presumptive diagnosis of sarcoidosis was made. She was, therefore, started on prednisolone and showed considerable improvement in her respiratory function tests.



FIGURE 1. Sarcoid of nose.

Past history. Fifteen years ago she had suffered from nasal stuffiness for which no cause was found. Nine years previously she had been investigated for a purulent cough and X-rays had shown irregular masses in both lungs. A firm diagnosis was not made, but a tuberculin test was negative.

Investigations. Haemoglobin 13.3 g/100 ml. E.S.R. 12 mm/h; W.B.C. 3700/mm³. X-rays of nasal bones: 'There has been partial absorption of the nasal bones, this appears to have been a long-standing process with no evidence of active destruction.' Biopsy of the palate (24/76) 'Sections show fragments of squamous epithelium and underlying sub-mucosa in which there is an epithelioid and giant cell

reaction. No acid fast bacilli are seen. These appearances taken in conjunction with the lymph node biopsy 24/76 are consistent with tuberculosis' (Dr P.D.James). Biopsy of supra-clavicular node (24/76) 'Sections of lymph node show loss of normal architecture and central replacement by acellular hyalinized tissue surrounded by an epithelioid and giant cell reaction. No acid fast bacilli are seen. The appearances are strongly suggestive of tuberculosis (Dr P.D.James).

Treatment and progress. There has been no further deterioration in her nose over the past 2 years.

Comment. The diagnosis lies between tuberculosis, Wegener's granulomatosis, Stuart's mid-line granuloma and sarcoidosis. There are features against all these, but it is felt that sarcoidosis is the most likely possibility although destruction of the nasal cartilage is not usual.

(26) Acne due to congenital adrenal hyperplasia

DR E.D.A.MITCHELL FOR DR B.R.ALLEN AND DR D.I.JOHNSTON

Children's Hospital, Nottingham

L.G., female, aged 4½ years. Hospital case no. 361799.

History. She was referred by the Child Health Department in January 1978 with acneiform spots on the face, chest and back since the age of 18 months. Her mother had recently noted pubic hair.

Past history. In 1976 at the age of 2 she was admitted to the Children's Hospital with broncho-

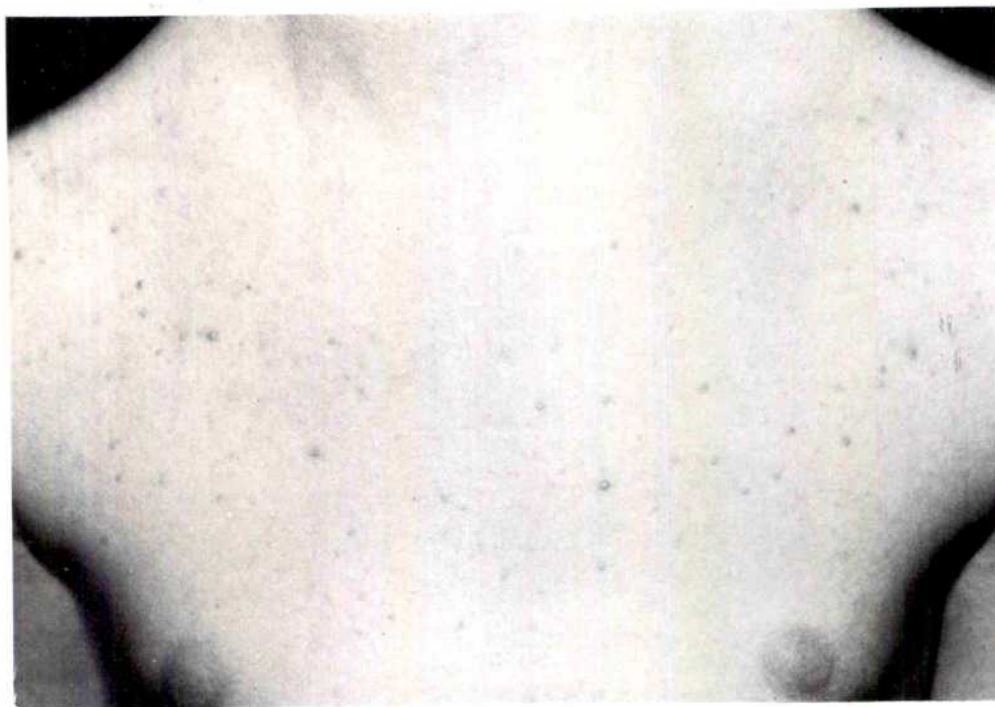


FIGURE 1. Acne of chest.

pneumonia. A blotchy, erythematous rash was noted on her trunk but there was no mention of acne. Her weight was around the 50th percentile for her age.

Family history. Nil relevant.

Examination. She is above the 97th percentile for both height and weight. She has acne of the face, back and chest, and greasy hair. Pubic hair is present and is associated with clitoral and labial hypertrophy.

When she was first seen, her blood pressure was 200/140 mmHg; this settled to 160/80 mmHg on admission to hospital.



FIGURE 2. Appearance of genitalia.

Investigations. Haemoglobin 11.4 g/100 ml; W.B.C. 8500/mm³; E.S.R. 6 mm/h; sodium 138 mmol/l; potassium 3.8 mmol/l; urea 2.4 mmol/l; urinary 17-oxogenic steroid 19.9 (normal 2.7); morning cortisol 966 (normal 280–700 mmol/l); morning ACTH 500 mg/l (normal up to 80); 17 α -hydroxyprogesterone 32.7 (normal up to 6).

Dexamethasone suppression test. High levels of urinary 17-oxogenic steroids were easily suppressed with both low and high doses of dexamethasone.

The above results are consistent with the diagnosis of congenital adrenal hyperplasia due to 11 β -hydroxylase deficiency. Chest X-ray normal. I.V.P. no significant abnormality.

X-ray of wrist for bone age. There is gross advance of skeletal maturation with well formed pisiform and sesamoid bones for the thumb. The epiphyseal plates are closing and there is differentiation of the carpals. Standard 19 (age 11).

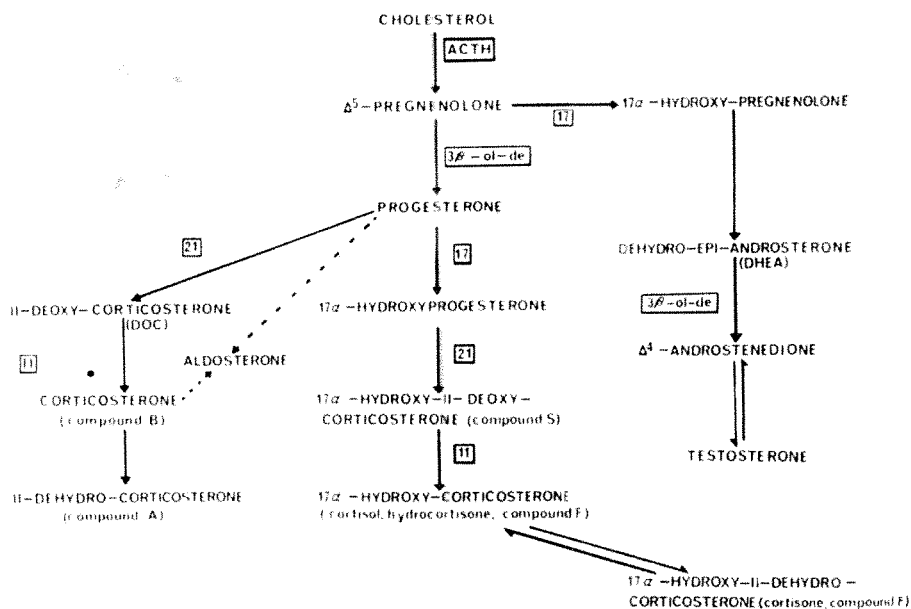


FIGURE 3. Pathways of steroid metabolism. II 17 21 = hydroxylases.
3 β -ol-de = 3 β -ol-dehydrogenase.

Reproduced by kind permission from *Practical Paediatric Problems* (3rd edn. 1972) by Professor J. H. Hutchinson, C.B.E., M.D. (London): Lloyd Luke ((Medical Books), Fig. 76.

Treatment. She has been started on hydrocortisone 30 mg daily and cyproterone acetate to delay precocious puberty.

Comment. Congenital adrenal hyperplasia is associated with over-production of adrenal androgens which results in virilization of the female. A deficiency of 11-hydroxylase results in excessive production of 17 α -hydroxy-11-deoxy corticosterone (compound S), 17 α -hydroxyprogesterone and of 11-deoxycorticosterone which is a potent mineralocorticoid with salt-retaining properties and a cause of hypertension.

(27) Keratis pilaris atrophicans

DR E.D.A. MITCHELL FOR DR B.R. ALLEN

Children's Hospital, Nottingham

R.T., female, aged 2½ years. Hospital case no. 156108.

History. Since her birth, her parents have noticed that she has 'rough-skin'. There has been very little change in the extent or severity of the disorder.

Family history. There is mild atopy on the paternal side but no eczema or other skin disease. A younger child is unaffected.

Examination. There are milia-like lesions with a follicular distribution on the cheeks and limbs. The involved areas are well demarcated.

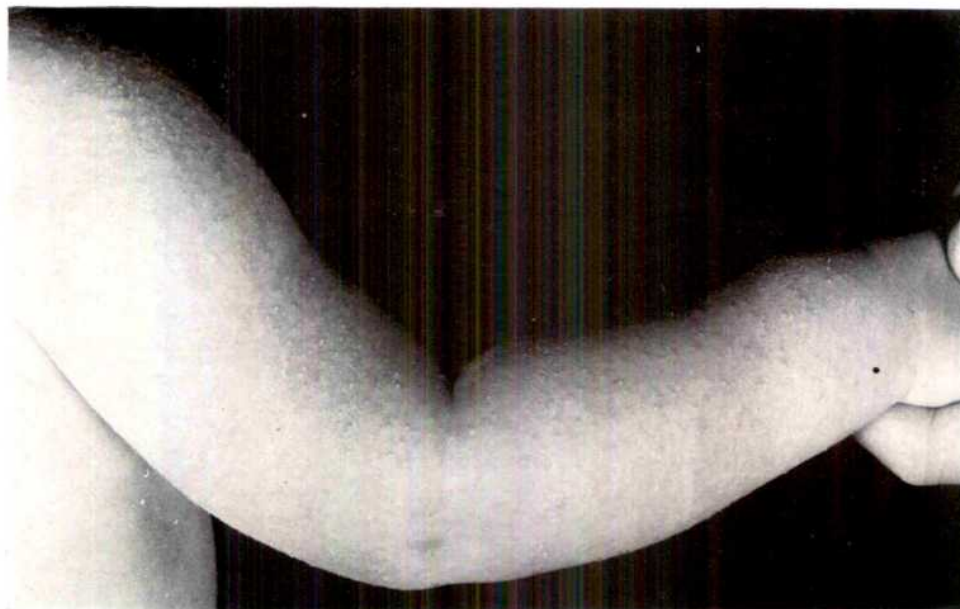


FIGURE 1. Right arm showing follicular lesions.

Histology. Biopsy from the left elbow: 'The only significant abnormality of the biopsy is in the hair follicles. Two were seen which were dilated in the middle third and filled with keratin. A third is seen in which the middle third of the follicle is destroyed by giant cell and epithelioid reactions. The appearances are that of a destructive folliculitis' (Dr P. D. James).

Treatment. Systemic vitamin A and Septrin and topical retinoic acid and hydrocortisone have been ineffective.

Comment. This girl's condition is obviously disfiguring and her parents would appreciate advice on prognosis.

(28) Collodion baby: ichthyosiform erythroderma

Dr A.E. WALKER

Royal Hospital, Chesterfield

S.T., male, aged 2 years. Hospital case No. 249081

History. This is the first child of unrelated parents. He was full term and the birth normal, but the skin changes consisting of severe ectropion, eversion of the lips and deep fissures around the neck, axillae and groins, were immediately apparent. His hands and feet were fixed in flexion. Within 4 days a membrane was shed and on the 10th day a small solitary blister was seen on the left buttock. He was discharged home on the 20th day.

Examination. He is a lively 2-year-old with erythema and ichthyosis of the extensor surfaces. There is also mild eczema of the flexures and he suffers intermittent napkin dermatitis. His scalp is rather scaly and hair thin. There are no blisters.



FIGURE 1. Appearance on the second day.

(29) Pemphigus vulgaris

DR A E. WALKER

Royal Hospital, Chesterfield

E.N., female, aged 15 years. Hospital case No. 251519.

History. She was referred in March, 1976 with a 6 week history of blisters and ulcers in the mouth and a 2 week history of similar lesions on the backs of the hands. There was no history of preceding illness or drug ingestion.

Examination. There is severe ulceration of the mouth and vulva. She has flaccid bullae and extensive areas of ulceration scattered over the trunk and limbs.

Investigation. Desmosome antibody positive 1:20. Skin biopsy shows an extensive intra-epidermal bulla with acantholysis and virtually no inflammatory reaction in the dermis. Direct immunofluorescence shows IgG and C3 deposition in the intercellular spaces of the epidermis of both involved and uninvolved skin.

Treatment. She was started on prednisolone 100 mg daily and azathioprine 50 mg b.d. and good control was obtained within 5 days. In September 1976 she was on prednisolone 12.5 mg daily and azathioprine 50 mg daily. Several investigations were repeated. Desmosome antibody was negative and direct immunofluorescence of the skin showed no IgG or C3. The dose of prednisolone was reduced to 5 mg daily and azathioprine was stopped. Blisters recurred within 3 weeks and prednisolone was increased and azathioprine reintroduced. The patient has since remained free of blisters and her present therapy is prednisolone 10 mg daily and azathioprine 50 mg daily.

(30) Xeroderma pigmentosum—a unique variant with neurological involvement

DR M.J.CHEESBROUGH FOR DR P.D.C.KINMONT

General Hospital, Nottingham

J.B., female, aged 17 years. Hospital case no. 168656.

History. Facial photosensitive erythema was noticed at age 3 months and blistering on the exposed parts at 5 months. She has developed freckles since but not tumours of the skin. She first walked at 18 months but was otherwise normal physically until the age of 11 when she became unsteady on her feet. Since then she has developed progressive pes cavus deformity. Mentally she has deteriorated since the age of about 11.

Family history. There is no consanguinity and no other known affected individuals.

Examination. She is small with bird-like facies. Her teeth, eyes and nails are normal. There is freckling, telangiectasia, pigmentation and atrophy on the exposed parts. On the covered areas there are pigmented naevi and a linear epidermal naevus on the mid line of the abdomen. She shows arachnodactyly (X-ray shows metacarpal index 9.34, upper limit of normal 8.0). Sexual development is normal.

Neurological examination shows microcephaly with mental retardation (mental age about 8). She has an intention tremor of both arms, ataxia of the legs, moderate spasticity of arms and legs and slight wasting of the small muscles of both hands. There is bilateral pes cavus producing a tendency to go up on the toes and flexion of both hips on walking. She has a wide based gait. There are no posterior column signs. Dr D.H.Mellor, paediatric neurologist, feels these signs indicate progressive spinocerebellar degeneration, cerebral degeneration and minimal polyneuropathy.

Investigations. E.M.G. showed changes suggesting partial denervation and the motor conduction velocity in the right ulnar nerve was just below the lower limit of normal. Excision repair in fibroblasts following U.V. irradiation 10–15%, normal (Dr A.M.R.Taylor, Department of Cancer Studies, Medical School, Birmingham). Investigation of fibroblasts by Dr Bootsma in Rotterdam showed that these cells are able to complement cells in all the previously known groups.

Comment. This patient is, therefore, genetically different from all previous X.P. patients examined and she has been allotted to the new complementation group G.

(31) Disseminated superficial actinic porokeratosis (DSAP)

DR E.N.MITCHELL JOHNSTON

General Hospital, Nottingham

L.C.G., female, aged 63 years. Hospital case no. 128135 GH.

History. For about 10 years circular, initially itchy, red lesions have been appearing on the shins with a few on the calves and forearms. They slowly enlarge to 5–10 mm diameter and finally resolve to leave a circular pale mark. They have not been noted to be more evident in English summers. No relevant medical, past, family or drug history. No response to local steroid ointments.

Examination. On the shins there are numerous circular, red and skin-coloured macules and papules with a fine thread-like keratotic border.

Histology. A cornoid lamella with basophilic fragments is seen arising from an epidermal notch, from which the granular layer is absent.

Investigations. W.R. and mycology negative.

Comment. Only half DSAP cases show accentuation of lesions in summer. One third complain of pruritus.

REFERENCE

CHERNOSKY, M.E. & FREEMAN, R.G. (1967) Disseminated superficial actinic porokeratosis (DSAP). *Archives of Dermatology*, **96**, 611.

(32) Porokeratosis of Mibelli

DR M.J.CHEESBROUGH FOR P.D.C.KINMONT

General Hospital, Nottingham

M.C., female, aged 17 years. Hospital case no. 308410 GH.

History. She has had a lesion on the right heel most of her life. It tends to crack and become painful.



FIGURE 1. Right heel.

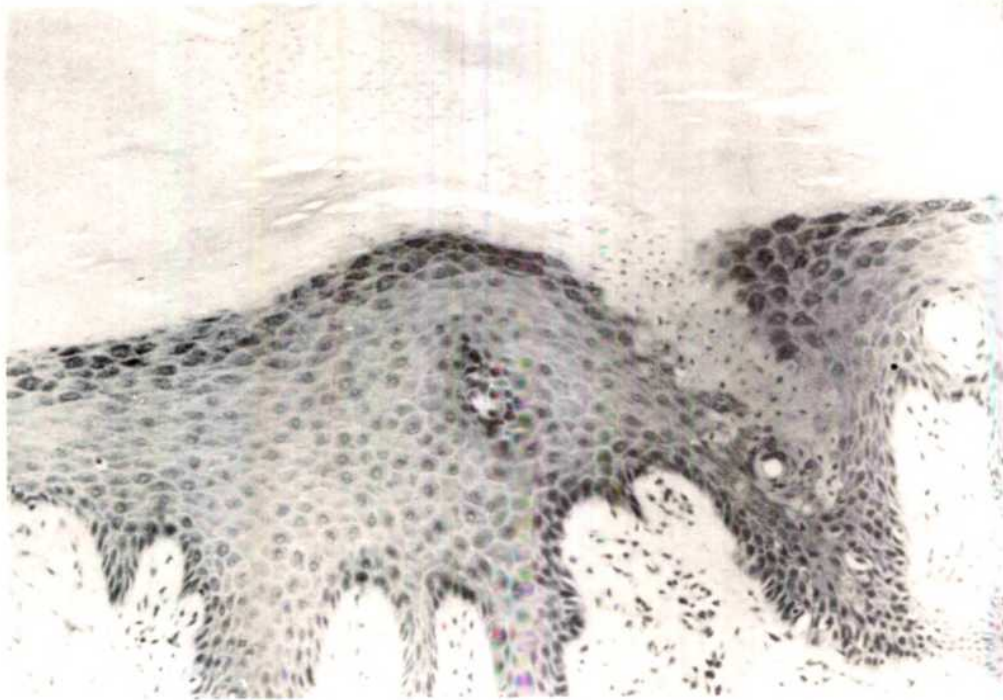


FIGURE 2. Epidermis showing cornoid lamella.

Examination. There is an irregular patch of hyperkeratosis on the plantar and posterior surface of the right heel. It has a well-defined and serpiginous edge.

Investigations. Mycology—negative. Histology shows the cornoid lamella of porokeratosis (7378/77).

(33) Cryoglobulinaemia

DR R.B.FOUNTAIN

Lincoln County Hospital, Lincoln

E.M.B., female, aged 67 years. Hospital case no. 73/16120.

History. In 1965 she first noticed areas of purpura on her legs and arms which blistered, became necrotic and ulcerated. These occurred in cold weather. This tendency has slowly become more severe, progressively involving more proximal parts of her limbs and also her nose and ears; even a cold night in the summer can now produce ulceration. Following an episode in 1972, in which 70% of her legs and 30% of her arms suffered full thickness necrosis, she has remained in hospital in a specially heated room. During this severe attack she very nearly died. Constant vigilance is required. Even a 2 min visit to a cold lavatory whilst having a barium enema resulted in an area of skin necrosis, nausea and malaise. Apart from her skin disease she suffers from endogenous depression and diverticulitis.

Examination. Extensive areas of pigmentation and scarring, often in a reticular pattern, cover most of her limbs, face and parts of her trunk. General examination is otherwise normal with no lymphadenopathy or enlargement of liver or spleen.

Investigations. Haemoglobin varies between 14.0 g/100 ml and 9.0 g/100 ml. W.B.C. normal; E.S.R. between 126 and 1 mm/h. Bone marrow—20% of nucleated cells were plasma cells. Skeletal survey normal. Urine—no Bence-Jones protein. Plasma proteins: cryoglobulin level 9.0 g/l rising to present 19 g/l. These commence precipitating at 25 °C. Electrophoresis: total protein 52 g/l; albumin 22 g/l; alpha 1 globulin 20 g/l; alpha 2 globulin 13 g/l; beta globulin 6.0 g/l; gamma globulin 1.0 g/l; a compact band comprised 6 g/l of gamma globulin. Immunoglobulins: IgA 240 mg%; IgG 1100 mg%; IgM 26 mg%; monoclonal IgG (lambda type) 600 mg% of total gamma globulin. A.N.F. and rheumatoid factor negative.

Treatment. Plasmapheresis at Addenbrooke's Hospital on two occasions produced no improvement. Cyclophosphamide in doses sufficient to depress the white count has not lowered the cryoglobulin level. Steroids were of no avail. She now remains in a room, the temperature of which is kept above 25 °C and remains comfortable.

(34) Severe necrotizing vasculitis, with cryoglobulinaemia, macroglobulinaemia and rheumatoid disease

DR T.B.WALLINGTON FOR DR W.G.REEVES

University Hospital, Nottingham

C.H. male, aged 51 years, retired policeman. Hospital case no. 274134.

History. He was admitted as a medical emergency severely ill. Over 9 weeks he had become increasingly tired and weak and developed a rash on his trunk and limbs. For 3 weeks he had been feverish with a productive cough, haemoptysis and diarrhoea. He was then receiving ibuprofen and cotrimoxazole.

Past history. Sero-positive polyarthritis since 1968. In 1974 this was complicated by albuminuria with microscopic haematuria and a macular skin rash, with some haemorrhagic areas. This resolved after stopping treatment with indomethacin and switching from naproxen to alopurinol. A head injury in 1950 was followed by mild epilepsy and treated with mysoline and phenobarbitone.

Family history. One sister had rheumatoid disease.

Examination. Pale and unwell, temperature 39.0 °C. A haemorrhagic rash involved his trunk and limbs. There was axillary lymphadenopathy and tender hepatomegaly. He had oral candidiasis and his optic fundi showed marked venous dilatation with retinal haemorrhages. His joints showed rheumatoid changes most marked in the hands and knees.

Investigations. Haemoglobin 4.5 g/dl, leukocytes $12.1 \times 10^9/l$, platelets $259 \times 10^9/l$, blood film showed grossly distorted red blood cells, bone marrow atypical but suggesting megaloblastic change. Prothrombin Index normal. Urea 23.3 mmol/l. Blood cultures sterile. Serum complement C3 47 mg/100 ml; C4 6 mg/100 ml (both normal).

Treatment and progress. He was managed initially by blood transfusion and antibiotics but his condition deteriorated with renal failure, requiring peritoneal dialysis, and cardiac failure. His skin rash also worsened particularly where ice packs had been applied to his back.

Further investigation. Immunological investigation showed: IgA 340 mg/100 ml; IgG 620 mg/100 ml; IgM considerably raised at 1900 mg/100 ml. Immuno-electrophoresis showed changes suggesting the presence of monoclonal IgM (Kappa). No urinary light chains were detected. Cryoglobulins were detected to a cryocrit of 35% composed of IgM and IgG as major components. Immuno-electrophoresis of the cryoglobulin also suggested the presence of monoclonal IgM Kappa.

Rheumatoid factor was present with a Rose-Waaler titre of 32. This was much lower than the titre

of 512 found when rheumatoid disease was originally diagnosed. A similar titre has been maintained through follow up. No other auto-antibodies were detected.

C3 and C4 levels remained low and strongly positive circulating anti-complementary activity was detected. The viscosity was raised at 1.91 at 37°C and increased further at lower temperatures.

Histology. Biopsy of a haemorrhagic lesion showed leukocytoclastic vasculitis with necrosis. Immunofluorescent examination showed IgA, IgG, IgM, C3, C1q and fibrin deposited in dermal vessels.

Further treatment and progress. Following diagnosis of mixed cryoglobulinaemia he was treated with cyclophosphamide and prednisone and by intensive plasma exchange. Plasma exchange lowered his cryocrit with accompanying clinical improvement.

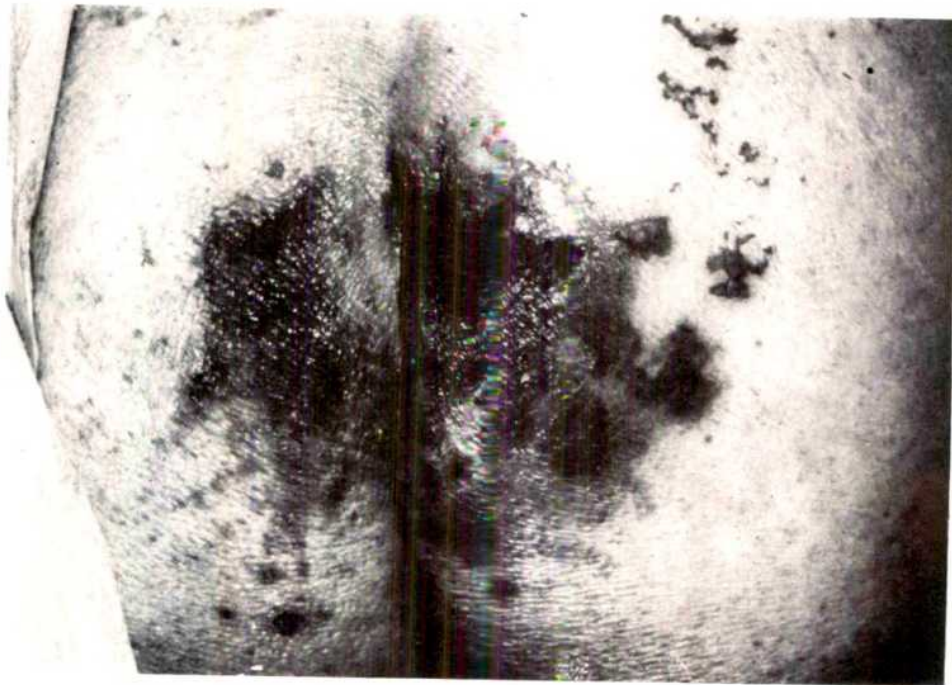


FIGURE 1. Haemorrhagic necrosis of the buttocks after the application of ice packs.

Eventually it proved possible to maintain him on small daily doses of chlorambucil and prednisone. Cryoglobulins are no longer detectable although anticomplementary activity is. Immunoglobulin levels are normal and 'monoclonal' IgM no longer detected. Lymphadenopathy had disappeared before he was well enough for a biopsy to be performed.

Comment. It is presumed that this patient's necrotizing vasculitis was due to the deposition of complement-fixing cryoglobulins in blood vessel walls. Removal of cryoglobulins improved his clinical condition when he was close to death and thus is an example of plasma exchange as a life-saving measure. Some evidence is provided for the cryoglobulins being of type II with a monoclonal IgM anti-IgG component. Attempts were made to separate this material into its two components by gel filtration at acid pH and high ionic strength at 37°C. However, the separated protein tended to precipitate spontaneously and it is still, largely on circumstantial grounds, that this is classified as type II cryoglobulinaemia.

REFERENCE

BROUET, J.-C., CLAUVEL, J.-P., DANON, F., KLEIN, M. & SELIGMANN, M. (1974) Biologic and clinical significance of cryoglobulins. A report of 86 cases. *American Journal of Medicine*, **57**, 775.

(35) Poikiloderma congenitale (Thomson-Rothmund syndrome)

DR M.J.CHEESBROUGH FOR DR P.D.C.KINMONT

General Hospital, Nottingham

J.B., female, aged 17 years. Hospital case no. 65138.

History. Erythema with oedema was first noted at the age of 6 months affecting the face, lower arms and hands, buttocks and legs. As she grew older it was noticed that her skin blistered on exposure to sun or cold winds and the lesions, particularly on the hands, feet and face, progressed to reticulate

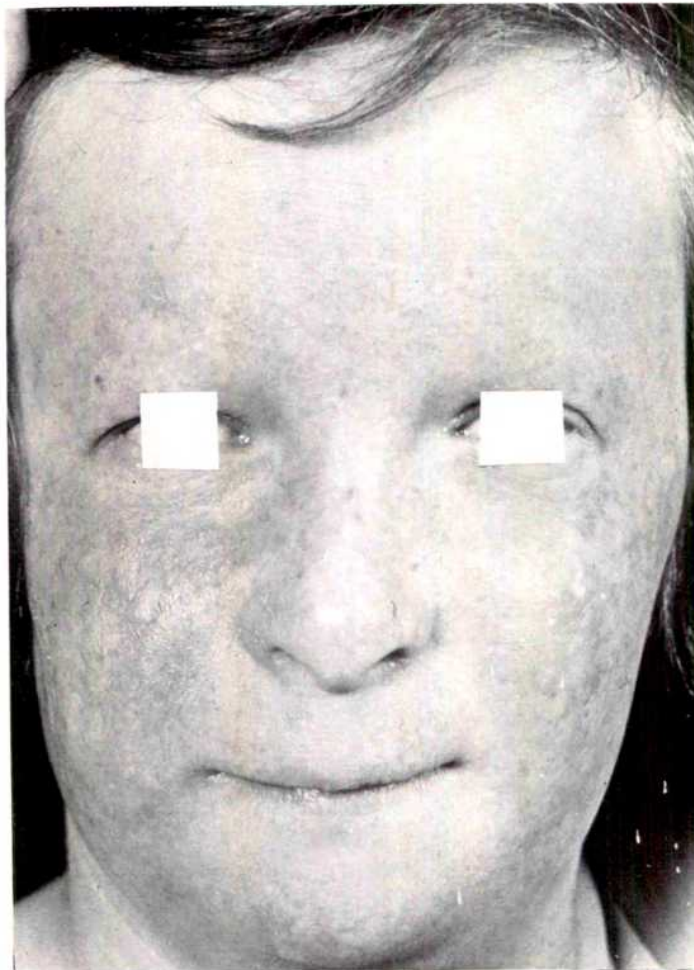


FIGURE 1. Face showing poikiloderma and absent eyebrows.

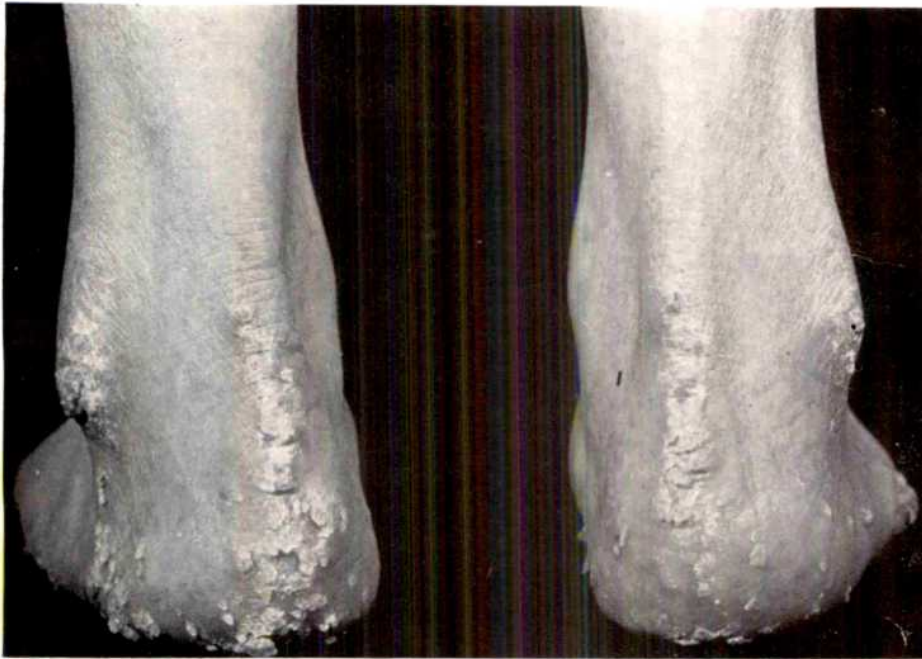


FIGURE 2. Keratoses on feet.

atrophy with irregular telangiectasia and pigmentation. At about the age of 4 it was noted that she had tender swellings on the outside of the left thigh, possibly due to small abscesses or chronic cystic areas due to low grade infection. Excision was considered but rejected on the grounds that a skin graft would be required. Soft tissue calcification was noted radiologically but investigations of calcium, phosphorous and alkaline phosphatase were normal in 1964 and again in 1972. She has developed keratoses on the hands and feet since 1970. Since the age of 2 she has been under the care of the orthopaedic surgeons when she was noted to have a dislocation of the head of the left radius. In 1971 she had a fracture of the middle third of the right tibia, possibly also of the 5th metatarsal of the left foot. She was noted to have a stress fracture of the left tibia in 1971. Ophthalmological examination in 1968 showed no evidence of cataract. Mental development has been normal. Menstruation started at the age of 13 and has since been normal.

Family history. There is no consanguinity and there is no relevant family history.

Examination. The skin shows striae, atrophy and pigmentation on the face and backs of hands and to a lesser extent on the arms and legs. Eyebrows and eyelashes are absent. There are multiple keratoses over the knuckles, left elbow scar, sides of feet, backs of heels and between the toes. The hands show incurving little fingers and thumbs laterally placed. Dentition and breast development are normal as are the other systems.

Investigations. Skin biopsy (446/64) showed moderate atrophy of the epidermis and slight reactive hyperkeratosis. Melanin present in the superficial dermis in occasional foci but no cellular infiltration (Dr G.F.M.Hall). Bone biopsy (Great Ormond Street, 1965) showed marrow cavity filled with dense fibrous material but no fat and no marrow tissue. The following investigations have been negative or normal—urine aminoacids, urine test for phenyl ketonuria, urine porphyrins and porphobilinogen, blood count, calcium, phosphorus, alkaline phosphatase, proteins and electrophoresis, blood sugar, chromosomes.

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